

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :  C12N 15/00, 15/11, 15/12 A61K 37/02, 37/04		A1	(11) International Publication Number: <b>WO 93/14201</b>  (43) International Publication Date: 22 July 1993 (22.07.93)
<p>(21) International Application Number: PCT/US93/00149</p> <p>(22) International Filing Date: 8 January 1993 (08.01.93)</p> <p>(30) Priority data: 07/817,920 8 January 1992 (08.01.92) US</p> <p>(60) Parent Application or Grant (63) Related by Continuation US 07/817,920 (CIP) Filed on 8 January 1992 (08.01.92)</p> <p>(71) Applicant (for all designated States except US): SYNAPTIC PHARMACEUTICAL CORPORATION [US/US]; 215 College Road, Paramus, NJ 07652 (US).</p>			
<p>(72) Inventors: and (75) Inventors/Applicants (for US only) : WEINSHANK, Richard, L. [US/US]; 302 West 87th Street, New York, NY 10024 (US). BRANCHEK, Theresa [US/US]; 541 Martense Avenue, Teaneck, NJ 07666 (US). HARTIG, Paul, R. [US/US]; 19 Pheasant Run, Kinnelon, NJ 07405 (US).</p> <p>(74) Agent: WHITE, John, P.: Cooper &amp; Dunham, 30 Rockefeller Plaza, New York, NY 10112 (US).</p> <p>(81) Designated States: AU, CA, FI, HU, JP, KR, NO, NZ, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published With international search report.</p>			

**(54) Title:** DNA ENCODING A HUMAN 5-HT<sub>1F</sub> RECEPTOR AND USES THEREOF**(57) Abstract**

This invention provides an isolated nucleic acid molecule encoding a human 5-HT<sub>1F</sub> receptor, an isolated protein which is a human 5-HT<sub>1F</sub> receptor, vectors comprising an isolated nucleic acid molecule encoding a human 5-HT<sub>1F</sub> receptor, mammalian cells comprising such vectors, antibodies directed to the human 5-HT<sub>1F</sub> receptor, nucleic acid probes useful for detecting nucleic acid encoding human 5-HT<sub>1F</sub> receptors, antisense oligonucleotides complementary to any sequences of a nucleic acid molecule which encodes a human 5-HT<sub>1F</sub> receptor, pharmaceutical compounds related to human 5-HT<sub>1F</sub> receptors, and nonhuman transgenic animals which express DNA a normal or a mutant human 5-HT<sub>1F</sub> receptor. This invention further provides methods for determining ligand binding, detecting expression, drug screening, and treatment involving the human 5-HT<sub>1F</sub> receptor.

Applicant: Douglas A. Craig  
J. S. Serial No.: 09/450,880  
Filed: November 29, 1999  
Group Art Unit: 1655

Exhibit 4

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SK	Slovak Republic
CI	Côte d'Ivoire	LJ	Liechtenstein	SN	Senegal
CM	Cameroon	LK	Sri Lanka	SU	Soviet Union
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	MC	Monaco	TG	Togo
DE	Germany	MG	Madagascar	UA	Ukraine
DK	Denmark	ML	Mali	US	United States of America
ES	Spain	MN	Mongolia	VN	Viet Nam
FI	Finland				

5 DNA ENCODING A HUMAN 5-HT<sub>1F</sub> RECEPTOR AND USES THEREOF10 Background of the Invention

Throughout this application various publications are referenced by partial citations within parentheses. The disclosures of these publications in their entireties are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains.

20 Since the purification of a pressor substance in blood serum termed serotonin (Rapport et al., 1947) and later identified as 5-hydroxytryptamine (5-HT) (Rapport, 1949), there has been a plethora of reports demonstrating that this indoleamine not only plays a role in the functioning of peripheral tissues but, indeed, performs a key role in the brain as a neurotransmitter. Certainly, the anatomical localization of serotonin and serotonergic neurons in both the peripheral and central nervous systems supports its role in such diverse physiologic and behavioral functions as pain perception, sleep, aggression, sexual activity, hormone secretion, thermoregulation, motor activity, cardiovascular function, food intake and renal regulation (For review see Green, 1985; Osborne and Hamon, 1988; Sanders-Bush, 1988; Peroutka, 1991). Taken together, it appears that serotonin plays an important role in homeostasis and in modulating responsiveness to environmental stimuli. Accordingly, studies demonstrating that abnormalities in the serotonergic system may be associated with disease states has created a drug development effort towards agents which may selectively modulate the function of serotonin (Glennon, 1990).

In relation to the characterization of physiologic or biochemical responses resulting from the release of serotonin are simultaneous investigations examining the receptor sites responsible for the actions elicited by the indoleamine transmitter. Following early in vitro pharmacological assays describing the existence of two different serotonin receptors, designated as D and M, in the guinea pig ileum (Gaddum and Picarelli, 1957), the advent of receptor binding technique in the 1970's has brought to light during the last decade the diversity of 5-HT receptors existing in both the brain and peripheral tissues. Thus, although the concept of D and M receptors has not been invalidated, serotonin receptors not fitting either category have been identified using radioligand methods. To date using this technique, there appears to be four classes of serotonin receptors found in the brain: 5-HT<sub>1</sub>, 5-HT<sub>2</sub>, 5-HT<sub>3</sub> and, putatively, 5-HT<sub>4</sub> (Peroutka, 1991). Furthermore, 5-HT<sub>1</sub> sites have been subclassified as: 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1C</sub>, 5-HT<sub>1D</sub> (Hamon et al., 1990) and 5-HT<sub>1E</sub> (Leonhardt et al., 1989). Although a detailed characterization of the 5-HT<sub>1F</sub> binding site is lacking, extensive pharmacologic, biochemical and functional properties have clearly shown that the other four subtypes of 5-HT<sub>1</sub> sites are receptors according to classical criteria.

During the last few years, the field of molecular biology has provided an important facet to receptor research by cloning these proteins and allowing more precise characterizations in isolated systems (Hartig et al., 1990). This has been accomplished for the 5-HT<sub>1A</sub> (Fargin et al., 1988), 5-HT<sub>1C</sub> (Julius et al., 1988), 5-HT<sub>1D</sub> (Branchek et al., 1990) and 5-HT<sub>2</sub> receptors (Pritchett et al., 1988). Thus, there is no doubt that these binding sites represent "true" functional receptors. Indeed, the pharmacological characterization of serotonin receptors involved in various physiological

or biochemical functions is a key component of drug development for the serotonergic system. As one can deduce from the diversity of serotonin binding sites, many targets are available for advancement in selective 5 drug design. The coupling of molecular biological methods to pharmacological characterization particularly for cloned human receptors will open new avenues for pharmaceutical development which has not been previously explored.

10

This study is a pharmacological characterization of a serotonergic receptor clone with a binding profile different from that of any serotonergic receptor to date. In keeping with the nomenclature presently accepted for 15 serotonin receptors, this novel site will be termed a 5-HT<sub>1F</sub> receptor based upon the fact that it possesses high affinity for the endogenous neurotransmitter, 5-HT.

Summary of the Invention

5 This invention provides an isolated nucleic acid molecule encoding a human 5-HT<sub>1F</sub> receptor (Seq. I.D. No. 1).

This invention also provides an isolated protein which is a human 5-HT<sub>1F</sub> receptor (Seq. I.D. Nos. 2, 7).

10 This invention provides a vector comprising an isolated nucleic acid molecule encoding a human 5-HT<sub>1F</sub> receptor.

15 This invention also provides vectors such as plasmids comprising a DNA molecule encoding a human 5-HT<sub>1F</sub> receptor, adapted for expression in a bacterial cell, a yeast cell, or a mammalian cell which additionally comprise the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cells so located relative to the DNA encoding the 5-HT<sub>1F</sub> receptor  
20 as to permit expression thereof.

This invention provides a mammalian cell comprising a DNA molecule encoding a human 5-HT<sub>1F</sub> receptor.

25 This invention provides a method for determining whether a ligand not known to be capable of binding to a human 5-HT<sub>1F</sub> receptor can bind to a human 5-HT<sub>1F</sub> receptor which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a human 5-HT<sub>1F</sub> receptor with the ligand under conditions permitting binding of ligands known to bind to a 5-HT<sub>1F</sub> receptor, detecting the presence of any of the ligand bound to a human 5-HT<sub>1F</sub> receptor, and thereby determining whether the ligand binds to a human 5-HT<sub>1F</sub> receptor.  
30

35 This invention also provides a method for determining whether a ligand not known to be capable of binding to

the human 5-HT<sub>1F</sub> receptor can functionally activate its activity or prevent the action of a ligand which does so. This comprises contacting a mammalian cell comprising an isolated DNA molecule which encodes a human 5-HT<sub>1F</sub> receptor with the ligand under conditions permitting the activation or blockade of a functional response, detected by means of a bioassay from the mammalian cell such as a second messenger response, and thereby determining whether the ligand activates or prevents the activation of the human 5-HT<sub>1F</sub> receptor functional output.

This invention further provides a method of screening drugs to identify drugs which specifically interact with, and bind to, the human 5-HT<sub>1F</sub> receptor on the surface of a cell which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a human 5-HT<sub>1F</sub> receptor with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, a human 5-HT<sub>1F</sub> receptor.

This invention also provides a method of screening drugs to identify drugs which interact with, and activate or block the activation of, the human 5-HT<sub>1F</sub> receptor on the surface of a cell which comprises contacting the mammalian cell comprising an isolated DNA molecule encoding and expressing a human 5-HT<sub>1F</sub> receptor with a plurality of drugs, determining those drugs which activate or block the activation of the receptor in the mammalian cell using a bioassay such as a second messenger assays, and thereby identifying drugs which specifically interact with, and activate or block the activation of, a human 5-HT<sub>1F</sub> receptor.

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence

included within the sequence of a nucleic acid molecule encoding a human 5-HT<sub>1F</sub> receptor.

This invention also provides a method of detecting expression of the 5-HT<sub>1F</sub> receptor on the surface of a cell by detecting the presence of mRNA coding for a 5-HT<sub>1F</sub> receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with a nucleic acid probe comprising a nucleic acid molecule of at least 5 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human 5-HT<sub>1F</sub> receptor under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the 10 15 expression of the 5-HT<sub>1F</sub> receptor by the cell.

This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a human 5-HT<sub>1F</sub> receptor so as to prevent translation of the 20 25 mRNA molecule.

This invention provides an antibody directed to a human 5-HT<sub>1F</sub> receptor.

25 This invention provides a transgenic nonhuman mammal expressing DNA encoding a human 5-HT<sub>1F</sub> receptor. This invention also provides a transgenic nonhuman mammal 30 expressing DNA encoding a human 5-HT<sub>1F</sub> receptor so mutated as to be incapable of normal receptor activity, and not expressing native 5-HT<sub>1F</sub> receptor. This invention further provides a transgenic nonhuman mammal whose genome 35 comprises antisense DNA complementary to DNA encoding a human 5-HT<sub>1F</sub> receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a 5-HT<sub>1F</sub> receptor and which hybridizes to mRNA encoding a 5-HT<sub>1F</sub> receptor thereby reducing its translation.

5 This invention provides a method of determining the physiological effects of expressing varying levels of human 5-HT<sub>1F</sub> receptors which comprises producing a transgenic nonhuman animal whose levels of human 5-HT<sub>1F</sub> receptor expression are varied by use of an inducible promoter which regulates human 5-HT<sub>1F</sub> receptor expression.

10 10 This invention also provides a method of determining the physiological effects of expressing varying levels of human 5-HT<sub>1F</sub> receptors which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human 5-HT<sub>1F</sub> receptor.

15 15 This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a specific human 5-HT<sub>1F</sub> receptor allele which comprises: a. obtaining DNA of subjects suffering from the disorder; b. performing a restriction digest of the DNA with a panel of restriction enzymes; c. 20 20 electrophoretically separating the resulting DNA fragments on a sizing gel; d. contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human 5-HT<sub>1F</sub> receptor and labelled with a detectable marker; e. detecting labelled bands which have hybridized to the DNA encoding a human 25 25 5-HT<sub>1F</sub> receptor labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f. preparing DNA obtained for diagnosis by steps a-e; and g. comparing the unique band pattern specific to the DNA of subjects 30 30 suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns 35 35 are the same.

This invention provides a method of preparing the

isolated 5-HT<sub>1F</sub> receptor which comprises inducing cells to express 5-HT<sub>1F</sub> receptor, recovering the receptor from the resulting cells and purifying the receptor so recovered.

5

This invention also provides a method of preparing the isolated 5-HT<sub>1F</sub> receptor which comprises inserting nucleic acid encoding 5-HT<sub>1F</sub> receptor in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the receptor produced by the resulting cell, and purifying the receptor so recovered.

10

This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a receptor so as to prevent translation of the mRNA molecule.

15

This invention also provides a transgenic nonhuman mammal expressing DNA encoding a receptor.

20

This invention further provides a transgenic nonhuman mammal expressing DNA encoding a receptor so mutated as to be incapable of normal receptor activity, and not expressing native receptor.

25

This invention also provides a method of determining the physiological effects of expressing varying levels of a receptor which comprises producing a transgenic nonhuman animal whose levels of receptor expression are varied by use of an inducible promoter which regulates receptor expression.

30

This invention also provides a method of determining the physiological effects of expressing varying levels of a receptor which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of

35

the receptor.

This invention further provides a transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding the receptor and which hybridizes to mRNA encoding the receptor thereby preventing its translation.

This invention provides a method for determining whether a ligand not known to be capable of binding to a receptor can bind to a receptor which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding the receptor with the ligand under conditions permitting binding of ligands known to bind to a receptor, detecting the presence of any of the ligand bound to the receptor, and thereby determining whether the ligand binds to the receptor.

Brief Description of the Figures

Figure 1. (Figures 1A-1F) Nucleotide and deduced amino acid sequence of gene 5-HT<sub>1F</sub> (Seq. I.D. Nos. 1, 2, and 7).

5 Numbers above the nucleotide sequence indicate nucleotide position. DNA sequence was determined by the chain termination method of Sanger, et al., on denatured double-stranded plasmid templates using the enzyme 10 Sequenase. Deduced amino acid sequence (single letter code) of a long open reading frame is shown.

15 Figure 2. (Figures 2A-2D) Comparison of the human 5-HT<sub>1F</sub> receptor primary structures with other serotonin receptors (Seq. I.D. Nos.: 5-HT<sub>1A</sub> - 3; 5-HT<sub>1C</sub> - 4; 5-HT<sub>1D</sub> - 5; 5-HT<sub>1DB</sub> - 6; 5-HT<sub>1F</sub> - 7; 5-HT<sub>2</sub> - 8).

20 Amino acid sequences (single letter code) are aligned to optimize homology. The putative transmembrane spanning domains are indicated by stars and identified by Roman numerals (TM I-VII).

25 Figure 3. 5-HT concentration-effect curves are represented in the absence (•) and in the presence (○) of methiothepin (1.0  $\mu$ M). Data were normalized to 100% relative to forskolin-stimulated values in the absence of agonist to derive values of  $E_{max}$  and  $E_{50}$ . The antagonist  $K_b$  was estimated by method of Furchtgott (32):  $K_b =$  30  $(\text{Dose of antagonist}) / ((E_{50} \text{ in the presence of antagonist}) / \text{control } E_{50}) - 1$ .

35 Figure 4. Human tissue distribution of RNA coding for 5-HT<sub>1F</sub> receptor gene. Total RNA was converted to single-stranded cDNA by random-priming with reverse transcriptase. cDNAs were amplified by PCR using 5-HT<sub>1F</sub> specific PCR primers. PCR products were run on a 1.5% agarose gel, blotted onto nylon membranes and hybridized

to internal gene-specific oligonucleotides and washed under high stringency. Positive controls represent gene-specific recombinant plasmids; dH<sub>2</sub>O served as a negative control. PCR amplification and Southern blotting of RNA samples not treated with reverse transcriptase were negative.

Figure 5 : 5-HT<sub>1F</sub> receptor mRNA in the guinea pig brain coronal sections. A. An antisense oligonucleotide probe (4,5 loop) was used. An identical pattern was observed with the 5' untranslated probe (not illustrated). Hybridization densities are high in layer V of cerebral cortex (V), and in CA1-CA3 of the hippocampus (HC). B. Control contralateral hemisphere of an adjacent section to that in A. No hybridization was seen using a sense probe of identical specific activity. C. Section hybridized with the antisense probe. The dorsal raphe (DR) is densely labeled. D. At high magnification, hybridization (antisense probe) is detected in layer V of sensorimotor cortex. Arrowheads indicate heavily labeled pyramidal cells. E. As in D, through the dorsal raphe. Arrowheads indicate large, heavily labeled neurons. Magnification in panels D and E = X270.

Detailed Description of the Invention

As used herein, the 5-HT receptor family is defined as the group of mammalian proteins that function as receptors for serotonin. A 5-HT receptor subfamily is defined as a subset of proteins belonging to the 5-HT receptor family which are encoded by genes which exhibit homology of greater than 72% or higher with each other in their deduced amino acid sequences within presumed transmembrane regions (linearly contiguous stretches of hydrophobic amino acids, bordered by charged or polar amino acids, that are long enough to form secondary protein structures that span a lipid bilayer). Four human 5-HT receptor subfamilies can be distinguished based on the information presently available: 5-HT<sub>1</sub>, 5-HT<sub>2</sub>, 5-HT<sub>3</sub>, and 5-HT<sub>4</sub> (Peroutka, 1991). The 5-HT<sub>2</sub> receptor subfamily contains the human 5-HT<sub>2</sub> receptor. Although no other human members of this family have been described, the rat 5-HT<sub>2</sub> receptor (Pritchett, et al. 1988; Julius, et al. Proc. Natl. Acad. Sci. USA 87:928-932, 1990) and the rat 5HT<sub>1C</sub> receptor (Julius, et al. 1988) constitute a rat 5-HT receptor subfamily. The 5-HT<sub>1</sub> subfamily has been subdivided further as: 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1C</sub>, 5-HT<sub>1D</sub> (Hamon et al., 1990) and 5-HT<sub>1F</sub> (Leonhardt et al., 1989). The 5-HT<sub>1A</sub> subfamily contains the human 5-HT<sub>1A</sub> receptor, also known as G-21 (Fargin, et al. 1988). The 5-HT<sub>1D</sub> receptor subfamily contains two members, the 5-HT<sub>1D-1</sub> receptor (also termed 5-HT<sub>1Dα</sub>) and the 5-HT<sub>1D-2</sub> receptor (also termed 5-HT<sub>1Dβ</sub>). The 5-HT<sub>1F</sub> subfamily contains the human 5-HT<sub>1F</sub> receptor (also termed clone h116a). Although this definition differs from the pharmacological definition used earlier, there is significant overlap between the present definition and the pharmacological definition. Members of the 5-HT<sub>1F</sub> receptor subfamily so described include the 5-HT<sub>1F</sub> receptor and any other receptors which have a greater

than 72% homology to the DNA and amino acid sequence shown in Figure 1 (Seq. I.D. Nos. 1, 2, and 7) according to the definition of "subfamily". This invention relates to the discovery of the first member of the human 5-HT<sub>1F</sub> receptor subfamily.

This invention provides an isolated nucleic acid molecule encoding a human 5-HT<sub>1F</sub> receptor (Seq. I.D. No. 1). As used herein, the term "isolated nucleic acid molecule" means a nucleic acid molecule that is, a molecule in a form which does not occur in nature. Such a receptor is by definition a member of the 5-HT<sub>1F</sub> receptor subfamily. Therefore, any receptor which meets the defining criteria given above is a human 5-HT<sub>1F</sub> receptor. One means of isolating a human 5-HT<sub>1F</sub> receptor is to probe a human genomic library with a natural or artificially designed DNA probe, using methods well known in the art. DNA probes derived from the human receptor gene 5-HT<sub>1F</sub> are particularly useful probes for this purpose. DNA and cDNA molecules which encode human 5-HT<sub>1F</sub> receptors may be used to obtain complementary genomic DNA, cDNA or RNA from human, mammalian or other animal sources, or to isolate related cDNA or genomic clones by the screening of cDNA or genomic libraries, by methods described in more detail below. Transcriptional regulatory elements from the 5' untranslated region of the isolated clones, and other stability, processing, transcription, translation, and tissue specificity-determining regions from the 3' and 5' untranslated regions of the isolated genes are thereby obtained. Examples of a nucleic acid molecule are an RNA, cDNA, or isolated genomic DNA molecule encoding a human 5-HT<sub>1F</sub> receptor. Such molecules may have coding sequences substantially the same as the coding sequence shown in Figure 1. The DNA molecule of Figure 1 encodes the sequence of the human 5-HT<sub>1F</sub> receptor gene (Seq. I.D. No. 1).

This invention further provides a cDNA molecule of encoding a human 5-HT<sub>1F</sub> receptor having a coding sequence substantially the same as the coding sequence shown in Figure 1 (Seq. I.D. No. 1). This molecule is obtained by 5 the means described above.

This invention also provides an isolated protein which is a human 5-HT<sub>1F</sub> receptor. As used herein, the term 10 "isolated protein means a protein molecule free of other cellular components. An example of such protein is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1 (Seq. I.D. Nos. 2, 7) which is a human 5-HT<sub>1F</sub> receptor. One means for obtaining isolated 5-HT<sub>1F</sub> receptor is to 15 express DNA encoding the receptor in a suitable host, such as a bacterial, yeast, or mammalian cell, using methods well known in the art, and recovering the receptor protein after it has been expressed in such a host, again using methods well known in the art. The receptor may also be isolated from cells which express 20 it, in particular from cells which have been transfected with the expression vectors described below in more detail.

25 This invention provides a vector comprising an isolated nucleic acid molecule such as DNA, RNA, or cDNA encoding a human 5-HT<sub>1F</sub> receptor. Examples of vectors are viruses such as bacteriophages (such as phage lambda), cosmids, 30 plasmids (such as pUC18, available from Pharmacia, Piscataway, NJ), and other recombination vectors. Nucleic acid molecules are inserted into vector genomes by methods well known in the art. For example, insert and vector DNA can both be exposed to a restriction 35 enzyme to create complementary ends on both molecules which base pair with each other and are then ligated together with a ligase. Alternatively, linkers can be ligated to the insert DNA which correspond to a

restriction site in the vector DNA, which is then digested with the restriction enzyme which cuts at that site. Other means are also available. A specific example of such plasmids is a plasmid comprising cDNA having a coding sequence substantially the same as the coding sequence shown in Figure 1 and designated clone h116a.

This invention also provides vectors comprising a DNA molecule encoding a human 5-HT<sub>1F</sub> receptor, adapted for expression in a bacterial cell, a yeast cell, or a mammalian cell which additionally comprise the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cells so located relative to the DNA encoding a human 5-HT<sub>1F</sub> receptor as to permit expression thereof. DNA having coding sequences substantially the same as the coding sequence shown in Figure 1 may usefully be inserted into the vectors to express human 5-HT<sub>1F</sub> receptors. Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG (Maniatis, et al., Molecular Cloning, Cold Spring Harbor Laboratory, 1982). Similarly, a eukaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors may be obtained commercially or assembled from the sequences described by methods well known in the art, for example the methods described above for constructing vectors in general. Expression vectors are useful to produce cells that express the receptor. Certain uses for such cells are described in more detail below.

This invention further provides a plasmid adapted for expression in a bacterial, yeast, or, in particular, a mammalian cell which comprises a DNA molecule encoding a human 5-HT<sub>1F</sub> receptor and the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cell so located relative to the DNA encoding a human 5-HT<sub>1F</sub> receptor as to permit expression thereof. Some plasmids adapted for expression in a mammalian cell are pSVL (available from Pharmacia, Piscataway, NJ), pcEXV-3 (Miller J. and Germain R.N., J. Exp. Med. 164:1478 (1986)) and pMO5 (Branchek, T. et al, Mol. Pharm. 38:604-609 (1990)). A specific example of such plasmid is a plasmid adapted for expression in a mammalian cell comprising cDNA having coding sequences substantially the same as the coding sequence shown in Figure 1 and the regulatory elements necessary for expression of the DNA in the mammalian cell which is designated pMO5-h116a and deposited under ATCC Accession No. 75175. Those skilled in the art will readily appreciate that numerous plasmids adapted for expression in a mammalian cell which comprise DNA of encoding human 5-HT<sub>1F</sub> receptors and the regulatory elements necessary to express such DNA in the mammalian cell may be constructed utilizing existing plasmids and adapted as appropriate to contain the regulatory elements necessary to express the DNA in the mammalian cell. The plasmids may be constructed by the methods described above for expression vectors and vectors in general, and by other methods well known in the art.

30

The deposit discussed supra, and the other deposits discussed herein, were made pursuant to, and in satisfaction of, the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852.

This invention provides a mammalian cell comprising a DNA molecule encoding a human 5-HT<sub>1F</sub> receptor, such as a mammalian cell comprising a plasmid adapted for expression in a mammalian cell, which comprises a DNA molecule encoding a human 5-HT<sub>1F</sub> receptor, the protein encoded thereby is expressed on the cell surface, and the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding a human 5-HT<sub>1F</sub> receptor as to permit expression thereof. Numerous mammalian cells may be used as hosts, including, for example, the mouse fibroblast cell NIH3T3, CHO cells, HeLa cells, Ltk<sup>-</sup> cells, Y1 cells, etc. A particular example of an Ltk<sup>-</sup> cell is a cell designated L-5-HT<sub>1F</sub> and deposited under ATCC Accession No. CRL 10957 and comprises the plasmid designated pM05-h116a. Another example is the murine fibroblast cell line designated N-5-HT<sub>1F</sub> and deposited under ATCC Accession No. CRL 10956. Expression plasmids such as that described supra may be used to transfect mammalian cells by methods well known in the art such as calcium phosphate precipitation, or DNA encoding these 5-HT<sub>1F</sub> receptors may be otherwise introduced into mammalian cells, e.g., by microinjection, to obtain mammalian cells which comprise DNA, e.g., cDNA or a plasmid, encoding either human 5-HT<sub>1F</sub> receptor.

This invention provides a method for determining whether a ligand not known to be capable of binding to a human 5-HT<sub>1F</sub> receptor can bind to a human 5-HT<sub>1F</sub> receptor which comprises contacting a mammalian cell comprising a DNA molecule encoding a human 5-HT<sub>1F</sub> receptor, the protein encoded thereby is expressed on the cell surface, with the ligand under conditions permitting binding of ligands known to bind to the 5-HT<sub>1F</sub> receptor, detecting the presence of any of the ligand bound to the 5-HT<sub>1F</sub> receptor, and thereby determining whether the ligand binds to the 5-HT<sub>1F</sub> receptor. This invention also provides a method for determining whether a ligand not

known to be capable of binding to the human 5-HT<sub>1F</sub> receptor can functionally activate its activity or prevent the action of a ligand which does so. This comprises contacting a mammalian cell comprising an isolated DNA molecule which encodes a human 5-HT<sub>1F</sub> receptor with the ligand under conditions permitting the activation or blockade of a functional response, detected by means of a bioassay from the mammalian cell such as a second messenger response, and thereby determining whether the ligand activates or prevents the activation of the human 5-HT<sub>1F</sub> receptor functional output. The DNA in the cell may have a coding sequence substantially the same as the coding sequence shown in Figure 1 preferably, the mammalian cell is nonneuronal in origin. An example of a nonneuronal mammalian cell is an Ltk<sup>-</sup> cell, in particular the Ltk<sup>-</sup> cell designated L-5-HT<sub>1F</sub>. Another example of a non-neuronal mammalian cell to be used for functional assays is a murine fibroblast cell line, specifically the NIH3T3 cell designated N-5-HT<sub>1F</sub>. The preferred method for determining whether a ligand is capable of binding to the human 5-HT<sub>1F</sub> receptor comprises contacting a transfected nonneuronal mammalian cell (i.e. a cell that does not naturally express any type of 5-HT or G-protein coupled receptor, thus will only express such a receptor if it is transfected into the cell) expressing a 5-HT<sub>1F</sub> receptor on its surface, or contacting a membrane preparation derived from such a transfected cell, with the ligand under conditions which are known to prevail, and thus to be associated with, in vivo binding of the ligands to a 5-HT<sub>1F</sub> receptor, detecting the presence of any of the ligand being tested bound to the 5-HT<sub>1F</sub> receptor on the surface of the cell, and thereby determining whether the ligand binds to, activates or prevents the activation of the 5-HT<sub>1F</sub> receptor. This response system is obtained by transfection of isolated DNA into a suitable host cell containing the desired second messenger system such as phosphoinositide

hydrolysis, adenylate cyclase, guanylate cyclase or ion channels. Such a host system is isolated from pre-existing cell lines, or can be generated by inserting appropriate components of second messenger systems into existing cell lines. Such a transfection system provides a complete response system for investigation or assay of the activity of human 5-HT<sub>1F</sub> receptors with ligands as described above. Transfection systems are useful as living cell cultures for competitive binding assays between known or candidate drugs and ligands which bind to the receptor and which are labeled by radioactive, spectroscopic or other reagents. Membrane preparations containing the receptor isolated from transfected cells are also useful for these competitive binding assays. Functional assays of second messenger systems or their sequelae in transfection systems act as assays for binding affinity and efficacy in the activation of receptor function. A transfection system constitutes a "drug discovery system" useful for the identification of natural or synthetic compounds with potential for drug development that can be further modified or used directly as therapeutic compounds to activate or inhibit the natural functions of the human 5-HT<sub>1F</sub> receptor. The transfection system is also useful for determining the affinity and efficacy of known drugs at the human 5-HT<sub>1F</sub> receptor sites.

This invention also provides a method of screening drugs to identify drugs which specifically interact with, and bind to, the human 5-HT<sub>1F</sub> receptor on the surface of a cell which comprises contacting a mammalian cell comprising a DNA molecule encoding a human 5-HT<sub>1F</sub> receptor on the surface of a cell with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, the human 5-HT<sub>1F</sub> receptor. This invention also provides a method of screening drugs to

identify drugs which interact with, and activate or block the activation of, the human 5-HT<sub>1F</sub> receptor on the surface of a cell which comprises contacting the mammalian cell comprising an isolated DNA molecule encoding and expressing a human 5-HT<sub>1F</sub> receptor with a plurality of drugs, determining those drugs which activate or block the activation of the receptor in the mammalian cell using a bioassay such as a second messenger assays, and thereby identifying drugs which specifically interact with, and activate or block the activation of, a human 5-HT<sub>1F</sub> receptor. The DNA in the cell may have a coding sequence substantially the same as the coding sequence shown in Figure 1 (Seq. I.D. No. 1). Preferably, the mammalian cell is nonneuronal in origin. An example of a nonneuronal mammalian cell is an Ltk<sup>-</sup> cell, in particular the Ltk<sup>-</sup> cell designated L-5-HT<sub>1F</sub>. Another example of a non-neuronal mammalian cell to be used for functional assays is a murine fibroblast cell line, specifically the NIH3T3 cell designated N-5-HT<sub>1F</sub>. Drug candidates are identified by choosing chemical compounds which bind with high affinity to the expressed 5-HT<sub>1F</sub> receptor protein in transfected cells, using radioligand binding methods well known in the art, examples of which are shown in the binding assays described herein. Drug candidates are also screened for selectivity by identifying compounds which bind with high affinity to one particular 5-HT<sub>1F</sub> receptor subtype but do not bind with high affinity to any other serotonin receptor subtype or to any other known receptor site. Because selective, high affinity compounds interact primarily with the target 5-HT<sub>1F</sub> receptor site after administration to the patient, the chances of producing a drug with unwanted side effects are minimized by this approach. This invention provides a pharmaceutical composition comprising a drug identified by the method described above and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically

acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. Once the candidate drug has been shown to be adequately bio-available following a particular route of administration, for example orally or by injection (adequate therapeutic concentrations must be maintained at the site of action for an adequate period to gain the desired therapeutic benefit), and has been shown to be non-toxic and therapeutically effective in appropriate disease models, the drug may be administered to patients by that route of administration determined to make the drug bio-available, in an appropriate solid or solution formulation, to gain the desired therapeutic benefit.

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human 5-HT<sub>1F</sub> receptor, for example with a coding sequence included within the sequence shown in Figure 1. As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. Detection of nucleic acid encoding human 5-HT<sub>1F</sub> receptors is useful as a diagnostic test for any disease process in which levels of expression of the corresponding 5-HT<sub>1F</sub> receptor is altered. DNA probe molecules are produced by insertion

of a DNA molecule which encodes human 5-HT<sub>1F</sub> receptor or fragments thereof into suitable vectors, such as plasmids or bacteriophages, followed by insertion into suitable bacterial host cells and replication and harvesting of the DNA probes, all using methods well known in the art. For example, the DNA may be extracted from a cell lysate using phenol and ethanol, digested with restriction enzymes corresponding to the insertion sites of the DNA into the vector (discussed above), electrophoresed, and cut out of the resulting gel. An example of such DNA molecule is shown in Figure 1. The probes are useful for 'in situ' hybridization or in order to locate tissues which express this gene family, or for other hybridization assays for the presence of these genes or their mRNA in various biological tissues. In addition, synthesized oligonucleotides (produced by a DNA synthesizer) complementary to the sequence of a DNA molecule which encodes human 5-HT<sub>1F</sub> receptor or are useful as probes for these genes, for their associated mRNA, or for the isolation of related genes by homology screening of genomic or cDNA libraries, or by the use of amplification techniques such as the Polymerase Chain Reaction. Synthesized oligonucleotides as described may also be used to determine the cellular localization of the mRNA produced by the 5-HT<sub>1F</sub> gene by in situ hybridization. An example of such an oligonucleotide is: 5'-TCTCACCACTCTCCAAAAGGACTTGGCCATTCACCTCCTCCTTG-3' (Seq. I.D. No. 9).

This invention also provides a method of detecting expression of a 5-HT<sub>1F</sub> receptor on the surface of a cell by detecting the presence of mRNA coding for a 5-HT<sub>1F</sub> receptor which comprises obtaining total mRNA from the cell using methods well known in the art and contacting the mRNA so obtained with a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence

included within the sequence of a nucleic acid molecule encoding a human 5-HT<sub>1F</sub> receptor under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the 5-HT<sub>1F</sub> receptor by the cell. Hybridization of probes to target nucleic acid molecules such as mRNA molecules employs techniques well known in the art. In one possible means of performing this method, nucleic acids are extracted by precipitation from lysed cells and the mRNA is isolated from the extract using a column which binds the poly-A tails of the mRNA molecules. The mRNA is then exposed to radioactively labelled probe on a nitrocellulose membrane, and the probe hybridizes to and thereby labels complementary mRNA sequences. Binding may be detected by autoradiography or scintillation counting. However, other methods for performing these steps are well known to those skilled in the art, and the discussion above is merely an example.

This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a human 5-HT<sub>1F</sub> receptor so as to prevent translation of the mRNA molecule. The antisense oligonucleotide may have a sequence capable of binding specifically with any sequences of the cDNA molecule whose sequence is shown in Figure 1. As used herein, the phrase "binding specifically" means the ability of a nucleic acid sequence to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. A particular example of an antisense oligonucleotide is an antisense oligonucleotide comprising chemical analogues of nucleotides.

35

This invention also provides a pharmaceutical composition comprising an amount of the oligonucleotide described

above effective to reduce expression of a human 5-HT<sub>1F</sub> receptor by passing through a cell membrane and binding specifically with mRNA encoding a human 5-HT<sub>1F</sub> receptor in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane. The oligonucleotide may be coupled to a substance which inactivates mRNA, such as a ribozyme. The pharmaceutically acceptable hydrophobic carrier capable of passing through cell membranes may also comprise a structure which binds to a receptor specific for a selected cell type and is thereby taken up by cells of the selected cell type. The structure may be part of a protein known to bind a cell-type specific receptor, for example an insulin molecule, which would target pancreatic cells. DNA molecules having coding sequences substantially the same as the coding sequence shown in Figure 1 may be used as the oligonucleotides of the pharmaceutical composition.

This invention also provides a method of treating abnormalities which are alleviated by reduction of expression of a 5-HT<sub>1F</sub> receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to reduce expression of the 5-HT<sub>1F</sub> receptor by the subject.

This invention further provides a method of treating an abnormal condition related to 5-HT<sub>1F</sub> receptor activity which comprises administering to a subject an amount of the pharmaceutical composition described above effective to reduce expression of the 5-HT<sub>1F</sub> receptor by the subject. Several examples of such abnormal conditions are dementia, Parkinson's disease, feeding disorders, pathological anxiety, schizophrenia, or a migraine headache.

35

Antisense oligonucleotide drugs inhibit translation of mRNA encoding these receptors. Synthetic

oligonucleotides, or other antisense chemical structures are designed to bind to mRNA encoding the 5-HT<sub>1F</sub> receptor and inhibit translation of mRNA and are useful as drugs to inhibit expression of 5-HT<sub>1F</sub> receptor genes in patients. This invention provides a means to therapeutically alter levels of expression of human 5-HT<sub>1F</sub> receptors by the use of a synthetic antisense oligonucleotide drug (SAOD) which inhibits translation of mRNA encoding these receptors. Synthetic oligonucleotides, or other antisense chemical structures designed to recognize and selectively bind to mRNA, are constructed to be complementary to portions of the nucleotide sequences shown in Figure 1 of DNA, RNA or of chemically modified, artificial nucleic acids. The SAOD is designed to be stable in the blood stream for administration to patients by injection, or in laboratory cell culture conditions, for administration to cells removed from the patient. The SAOD is designed to be capable of passing through cell membranes in order to enter the cytoplasm of the cell by virtue of physical and chemical properties of the SAOD which render it capable of passing through cell membranes (e.g. by designing small, hydrophobic SAOD chemical structures) or by virtue of specific transport systems in the cell which recognize and transport the SAOD into the cell. In addition, the SAOD can be designed for administration only to certain selected cell populations by targeting the SAOD to be recognized by specific cellular uptake mechanisms which binds and takes up the SAOD only within certain selected cell populations. For example, the SAOD may be designed to bind to a receptor found only in a certain cell type, as discussed above. The SAOD is also designed to recognize and selectively bind to the target mRNA sequence, which may correspond to a sequence contained within the sequence shown in Figure 1 by virtue of complementary base pairing to the mRNA. Finally, the SAOD is designed to inactivate the target mRNA sequence

by any of three mechanisms: 1) by binding to the target mRNA and thus inducing degradation of the mRNA by intrinsic cellular mechanisms such as RNase I digestion, 2) by inhibiting translation of the mRNA target by interfering with the binding of translation-regulating factors or of ribosomes, or 3) by inclusion of other chemical structures, such as ribozyme sequences or reactive chemical groups, which either degrade or chemically modify the target mRNA. Synthetic antisense oligonucleotide drugs have been shown to be capable of the properties described above when directed against mRNA targets (J.S. Cohen, Trends in Pharm. Sci. 10, 435 (1989); H.M. Weintraub, Sci. Am. January (1990) p. 40). In addition, coupling of ribozymes to antisense oligonucleotides is a promising strategy for inactivating target mRNA (N. Sarver et al., Science 247, 1222 (1990)). An SAOD serves as an effective therapeutic agent if it is designed to be administered to a patient by injection, or if the patient's target cells are removed, treated with the SAOD in the laboratory, and replaced in the patient. In this manner, an SAOD serves as a therapy to reduce receptor expression in particular target cells of a patient, in any clinical condition which may benefit from reduced expression of 5-HT<sub>1F</sub> receptors.

This invention provides an antibody directed to the human 5-HT<sub>1F</sub> receptor, for example a monoclonal antibody directed to an epitope of a human 5-HT<sub>1F</sub> receptor present on the surface of a cell and having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human 5-HT<sub>1F</sub> receptor included in the amino acid sequence shown in Figure 1 (Seq. I.D. Nos. 2, 7). Amino acid sequences may be analyzed by methods well known in the art to determine whether they produce hydrophobic or hydrophilic regions in the proteins which they build. In the case of cell membrane proteins, hydrophobic regions are well known to

form the part of the protein that is inserted into the lipid bilayer which forms the cell membrane, while hydrophilic regions are located on the cell surface, in an aqueous environment. Therefore antibodies to the hydrophilic amino acid sequences shown in Figure 1 will bind to a surface epitope of a human 5-HT<sub>1F</sub> receptor, as described. Antibodies directed to human 5-HT<sub>1F</sub> receptors may be serum-derived or monoclonal and are prepared using methods well known in the art. For example, monoclonal antibodies are prepared using hybridoma technology by fusing antibody producing B cells from immunized animals with myeloma cells and selecting the resulting hybridoma cell line producing the desired antibody. Cells such as NIH3T3 cells or Ltk<sup>-</sup> cells may be used as immunogens to raise such an antibody. Alternatively, synthetic peptides may be prepared using commercially available machines and the amino acid sequence shown in Figure 1. As a still further alternative, DNA, such as a cDNA or a fragment thereof, may be cloned and expressed and the resulting polypeptide recovered and used as an immunogen. These antibodies are useful to detect the presence of human 5-HT<sub>1F</sub> receptors encoded by the isolated DNA, or to inhibit the function of the receptors in living animals, in humans, or in biological tissues or fluids isolated from animals or humans.

This invention provides a pharmaceutical composition which comprises an amount of an antibody directed to the human 5-HT<sub>1F</sub> receptor effective to block binding of naturally occurring ligands to the 5-HT<sub>1F</sub> receptor, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to an epitope of a human 5-HT<sub>1F</sub> receptor present on the surface of a cell and having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human 5-HT<sub>1F</sub> receptor included in the amino acid sequence shown in Figure 1 is useful for this purpose.

This invention also provides a method of treating abnormalities which are alleviated by reduction of expression of a human 5-HT<sub>1F</sub> receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to block binding of naturally occurring ligands to the 5-HT<sub>1F</sub> receptor and thereby alleviate abnormalities resulting from overexpression of a human 5-HT<sub>1F</sub> receptor. Binding of the antibody to the receptor prevents the receptor from functioning, thereby neutralizing the effects of overexpression. The monoclonal antibodies described above are both useful for this purpose. This invention additionally provides a method of treating an abnormal condition related to an excess of 5-HT<sub>1F</sub> receptor activity which comprises administering to a subject an amount of the pharmaceutical composition described above effective to block binding of naturally occurring ligands to the 5-HT<sub>1F</sub> receptor and thereby alleviate the abnormal condition. Some examples of abnormal conditions are dementia, Parkinson's disease, feeding disorders, pathological anxiety, schizophrenia, and a migraine headache.

This invention provides a method of detecting the presence of a 5-HT<sub>1F</sub> receptor on the surface of a cell which comprises contacting the cell with an antibody directed to the human 5-HT<sub>1F</sub> receptor, under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby the presence of the human 5-HT<sub>1F</sub> receptor on the surface of the cell. Such a method is useful for determining whether a given cell is defective in expression of 5-HT<sub>1F</sub> receptors on the surface of the cell. Bound antibodies are detected by methods well known in the art, for example by binding fluorescent markers to the antibodies and examining the cell sample under a fluorescence microscope to detect fluorescence on a cell.

indicative of antibody binding. The monoclonal antibodies described above are useful for this purpose.

This invention provides a transgenic nonhuman mammal expressing DNA encoding a human 5-HT<sub>1F</sub> receptor. This invention also provides a transgenic nonhuman mammal expressing DNA encoding a human 5-HT<sub>1F</sub> receptor so mutated as to be incapable of normal receptor activity, and not expressing native 5-HT<sub>1F</sub> receptor. This invention also provides a transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a human 5-HT<sub>1F</sub> receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a 5-HT<sub>1F</sub> receptor and which hybridizes to mRNA encoding a 5-HT<sub>1F</sub> receptor thereby reducing its translation. The DNA may additionally comprise an inducible promoter or additionally comprise tissue specific regulatory elements, so that expression can be induced, or restricted to specific cell types. Examples of DNA are DNA or cDNA molecules having a coding sequence substantially the same as the coding sequence shown in Figure 1 (Seq. I.D. No. 1). An example of a transgenic animal is a transgenic mouse. Examples of tissue specificity-determining regions are the metallothionein promotor (Low, M.J., Lechan, R.M., Hammer, R.E. et al. Science 231:1002-1004 (1986)) and the L7 promotor (Oberdick, J., Smeyne, R.J., Mann, J.R., Jackson, S. and Morgan, J.I. Science 248:223-226 (1990)).

Animal model systems which elucidate the physiological and behavioral roles of human 5-HT<sub>1F</sub> receptors are produced by creating transgenic animals in which the expression of a 5-HT<sub>1F</sub> receptor is either increased or decreased, or the amino acid sequence of the expressed 5-HT<sub>1F</sub> receptor protein is altered, by a variety of techniques. Examples of these techniques include: 1) Insertion of normal or mutant versions of DNA encoding a

human 5-HT<sub>1F</sub> receptor or homologous animal versions of these genes, by microinjection, retroviral infection or other means well known to those skilled in the art, into appropriate fertilized embryos in order to produce a transgenic animal (Hogan B. et al. *Manipulating the Mouse Embryo, A Laboratory Manual*, Cold Spring Harbor Laboratory (1986)). 2) Homologous recombination (Capecchi M.R. *Science* 244:1288-1292 (1989); Zimmer, A. and Gruss, P. *Nature* 338:150-153 (1989)) of mutant or normal, human or animal versions of these genes with the native gene locus in transgenic animals to alter the regulation of expression or the structure of these 5-HT<sub>1F</sub> receptors. The technique of homologous recombination is well known in the art. It replaces the native gene with the inserted gene and so is useful for producing an animal that cannot express native receptor but does express, for example, an inserted mutant receptor, which has replaced the native receptor in the animal's genome by recombination, resulting in underexpression of the receptor. Microinjection adds genes to the genome, but does not remove them, and so is useful for producing an animal which expresses its own and added receptors, resulting in overexpression of the receptor. One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as M2 medium (Hogan B. et al. *Manipulating the Mouse Embryo, A Laboratory Manual*, Cold Spring Harbor Laboratory (1986)). DNA or cDNA encoding a human 5-HT<sub>1F</sub> receptor is purified from a vector (such as plasmid pMOS-h116a described above) by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the trans-gene. Alternatively or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific

expression of the trans-gene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA into the egg cell, and is used here only for exemplary purposes.

Since the normal action of receptor-specific drugs is to activate or to inhibit the receptor, the transgenic animal model systems described above are useful for testing the biological activity of drugs directed against these 5-HT<sub>1F</sub> receptors even before such drugs become available. These animal model systems are useful for predicting or evaluating possible therapeutic applications of drugs which activate or inhibit these 5-HT<sub>1F</sub> receptors by inducing or inhibiting expression of the native or trans-gene and thus increasing or decreasing expression of normal or mutant 5-HT<sub>1F</sub> receptors in the living animal. Thus, a model system is produced in which the biological activity of drugs directed against these 5-HT<sub>1F</sub> receptors are evaluated before such drugs become available. The transgenic animals which over or under produce the 5-HT<sub>1F</sub> receptor indicate by their physiological state whether over or under production of the 5-HT<sub>1F</sub> receptor is therapeutically useful. It is therefore useful to evaluate drug action based on the transgenic model system. One use is based on the fact that it is well known in the art that a drug such as an antidepressant acts by blocking neurotransmitter uptake,

and thereby increases the amount of neurotransmitter in the synaptic cleft. The physiological result of this action is to stimulate the production of less receptor by the affected cells, leading eventually to underexpression. Therefore, an animal which underexpresses receptor is useful as a test system to investigate whether the actions of such drugs which result in under expression are in fact therapeutic. Another use is that if overexpression is found to lead to abnormalities, then a drug which down-regulates or acts as an antagonist to 5-HT<sub>1F</sub> receptor is indicated as worth developing, and if a promising therapeutic application is uncovered by these animal model systems, activation or inhibition of the 5-HT<sub>1F</sub> receptor is achieved therapeutically either by producing agonist or antagonist drugs directed against these 5-HT<sub>1F</sub> receptors or by any method which increases or decreases the expression of these 5-HT<sub>1F</sub> receptors in man.

This invention provides a method of determining the physiological effects of expressing varying levels of human 5-HT<sub>1F</sub> receptors which comprises producing a transgenic nonhuman animal whose levels of human 5-HT<sub>1F</sub> receptor expression are varied by use of an inducible promoter which regulates human 5-HT<sub>1F</sub> receptor expression. This invention also provides a method of determining the physiological effects of expressing varying levels of human 5-HT<sub>1F</sub> receptors which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human 5-HT<sub>1F</sub> receptor. Such animals may be produced by introducing different amounts of DNA encoding a human 5-HT<sub>1F</sub> receptor into the oocytes from which the transgenic animals are developed.

This invention also provides a method for identifying a substance capable of alleviating abnormalities resulting from overexpression of a human 5-HT<sub>1F</sub> receptor comprising

5 administering the substance to a transgenic nonhuman mammal expressing at least one artificially introduced DNA molecule encoding a human 5-HT<sub>1F</sub> receptor and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overexpression of a human 5-HT<sub>1F</sub> receptor. As used herein, the term "substance" means a compound or composition which may be natural, synthetic, or a product derived from screening. Examples 10 of DNA molecules are DNA or cDNA molecules having a coding sequence substantially the same as the coding sequence shown in Figure 1.

15 This invention provides a pharmaceutical composition comprising an amount of the substance described supra effective to alleviate the abnormalities resulting from overexpression of 5-HT<sub>1F</sub> receptor and a pharmaceutically acceptable carrier.

20 This invention further provides a method for treating the abnormalities resulting from overexpression of a human 5-HT<sub>1F</sub> receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting 25 from overexpression of a human 5-HT<sub>1F</sub> receptor.

30 This invention provides a method for identifying a substance capable of alleviating the abnormalities resulting from underexpression of a human 5-HT<sub>1F</sub> receptor comprising administering the substance to the transgenic nonhuman mammal described above which expresses only nonfunctional human 5-HT<sub>1F</sub> receptor and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of underexpression of a human 35 5-HT<sub>1F</sub> receptor.

This invention also provides a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of 5-HT<sub>1F</sub> receptor and a pharmaceutically acceptable carrier.

5

This invention further provides a method for treating the abnormalities resulting from underexpression of a human 5-HT<sub>1F</sub> receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from underexpression of a human 5-HT<sub>1F</sub> receptor.

10

This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a specific human 5-HT<sub>1F</sub> receptor allele which comprises: a) obtaining DNA of subjects suffering from the disorder; b) performing a restriction digest of the DNA with a panel of restriction enzymes; c. electrophoretically separating the resulting DNA fragments on a sizing gel; d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human 5-HT<sub>1F</sub> receptor and labelled with a detectable marker; e) detecting labelled bands which have hybridized to the DNA encoding a human 5-HT<sub>1F</sub> receptor labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f) preparing DNA obtained for diagnosis by steps a-e; and g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and thereby to diagnose predisposition to the disorder if the patterns are the same. This method may also be used to diagnose a disorder associated with the expression of a specific human 5-HT<sub>1F</sub> receptor allele.

30

35

This invention provides a method of preparing the isolated 5-HT<sub>1F</sub> receptor which comprises inducing cells to express 5-HT<sub>1F</sub> receptor, recovering the receptor from the resulting cells, and purifying the receptor so recovered. An example of an isolated 5-HT<sub>1F</sub> receptor is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1 (Seq. I.D. Nos. 2, 7). For example, cells can be induced to express receptors by exposure to substances such as hormones. The cells can then be homogenized and the receptor isolated from the homogenate using an affinity column comprising, for example, serotonin or another substance which is known to bind to the receptor. The resulting fractions can then be purified by contacting them with an ion exchange column, and determining which fraction contains receptor activity or binds anti-receptor antibodies.

This invention provides a method of preparing the isolated 5-HT<sub>1F</sub> receptor which comprises inserting nucleic acid encoding 5-HT<sub>1F</sub> receptor in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the receptor produced by the resulting cell, and purifying the receptor so recovered. An example of an isolated 5-HT<sub>1F</sub> receptor is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1. This method for preparing 5-HT<sub>1F</sub> receptor uses recombinant DNA technology methods well known in the art. For example, isolated nucleic acid encoding 5-HT<sub>1F</sub> receptor is inserted in a suitable vector, such as an expression vector. A suitable host cell, such as a bacterial cell, or a eukaryotic cell such as a yeast cell, is transfected with the vector. 5-HT<sub>1F</sub> receptor is isolated from the culture medium by affinity purification or by chromatography or by other methods well known in the art.

This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a receptor so as to prevent translation of the mRNA molecule (Seq. I.D. No. 9).  
5

This invention also provides a transgenic nonhuman mammal expressing DNA encoding a receptor.

10 This invention further provides a transgenic nonhuman mammal expressing DNA encoding a receptor so mutated as to be incapable of normal receptor activity, and not expressing native receptor.

15 This invention provides a method of determining the physiological effects of expressing varying levels of a receptor which comprises producing a transgenic nonhuman animal whose levels of receptor expression are varied by use of an inducible promoter which regulates receptor expression.  
20

25 This invention also provides a method of determining the physiological effects of expressing varying levels of a receptor which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of the receptor.

30 This invention further provides transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding the receptor and which hybridizes to mRNA encoding the receptor thereby preventing its translation.

35 This invention provides a method for determining whether a ligand not known to be capable of binding to a receptor

can bind to a receptor which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding the receptor with the ligand under conditions permitting binding of ligands known to bind to a receptor, detecting the presence of any of the ligand bound to the receptor, and thereby determining whether the ligand binds to the receptor.

Applicants have identified individual receptor subtype proteins and have described methods for the identification of pharmacological compounds for therapeutic treatments. Pharmacological compounds which are directed against specific receptor subtypes provide effective new therapies with minimal side effects.

This invention identifies for the first time a new receptor protein, its amino acid sequence, and its human gene. Furthermore, this invention describes a previously unrecognized group of receptors within the definition of a 5-HT<sub>1F</sub> receptor. The information and experimental tools provided by this discovery are useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for this new receptor protein, its associated mRNA molecule or its associated genomic DNA. The information and experimental tools provided by this discovery will be useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for this new receptor protein, its associated mRNA molecule, or its associated genomic DNA.

Specifically, this invention relates to the first isolation of a human cDNA and genomic clone encoding a 5-HT<sub>1F</sub> receptor. A new human gene for the receptor identified herein as 5-HT<sub>1F</sub> has been identified and characterized, and a series of related cDNA and genomic clones have been isolated. In addition, the human 5-HT<sub>1F</sub> receptor has been expressed in Ltk<sup>-</sup> cells and NIH3T3

5           cells by transfecting the cells with the plasmid pMO5-h116a. The pharmacological binding properties of the protein encoded have been determined, and these binding properties classify this protein as a serotonin 5-HT<sub>1F</sub> receptor. Mammalian cell lines expressing this human 5-HT<sub>1F</sub> receptor at the cell surface have been constructed, thus establishing the first well-defined, cultured cell lines with which to study this 5-HT<sub>1F</sub> receptor.

10           The invention will be better understood by reference to the Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention as described more fully in the claims which  
15           follow thereafter.

## EXPERIMENTAL DETAILS

## Materials and Methods

Cloning and Sequencing: A human lymphocyte genomic library (Stratagene) was screened using the rat S51 fragment (obtained by PCR) as a probe. The probe was labeled with  $^{32}\text{P}$  by the method of random priming (Feinberg et al., 1983). Hybridization was performed at 50°C in a solution containing 50% formamide, 10% dextran sulfate, 5X SSC (1X SSC is 0.15 M sodium chloride, 0.015 M sodium citrate), 1X Denhardt's (0.02% polyvinylpyrrolidone, 0.02% Ficoll, and 0.02% bovine serum albumin), and 200  $\mu\text{g}/\text{ml}$  of sonicated salmon sperm DNA. The filters were washed at 50°C in 0.1X SSC containing 0.1% sodium dodecyl sulfate (SDS) and exposed at -70°C to Kodak XAR film in the presence of an intensifying screen. Lambda phage hybridizing to the probe were plaque purified and DNA was prepared for Southern blot analysis (Southern, 1975; Maniatis et al., 1982). For subcloning and further Southern blot analysis DNA was inserted into pUC18 (Pharmacia, Piscataway, N.J.). Nucleotide sequence analysis was done by the Sanger dideoxy nucleotide chain-termination method (Sanger 1977) on denatured double-stranded plasmid templates using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio).

Expression: The entire coding region of clone h116a was cloned into the eukaryotic expression vector pcEXV-3 (Miller, 1986). Stable cell lines were obtained by cotransfection with the plasmid pcEXV-3 (containing the 5-HT<sub>1F</sub> receptor gene) and the plasmid pGCCcos3neo (containing the aminoglycoside transferase gene) into Ltk<sup>-</sup> cells or NIH3T3 cells using calcium phosphate (reagents obtained from Specialty Media, Lavellette, NJ). The cells were grown in a controlled environment (37°C, 5% CO<sub>2</sub>) as monolayers in Dulbecco's modified Eagle medium (Gibco, Grand Island, N.Y.) containing 25 mM glucose and supplemented with 10% bovine calf serum, 100 U/ml penicillin G and 100  $\mu\text{g}/\text{ml}$  streptomycin sulfate. Stable clones were then selected for resistance to the

antibiotic G-418 and harvested membranes were screened for their ability to bind [<sup>3</sup>H]serotonin.

5                   Membrane Preparation: Membranes were prepared from transfected Ltk- cells which were grown to 100% confluence. The cells were washed twice with phosphate-buffered saline, scraped from the culture dishes into 5 ml of ice-cold phosphate-buffered saline, and centrifuged at 200 x g for 5 min at 4°. The pellet was resuspended in 2.5 ml of ice-cold Tris buffer (20 mM Tris -HCl, pH 10 7.4 at 23°, 5 mM EDTA) and homogenized by a Wheaton tissue grinder. The lysate was subsequently centrifuged at 200 x g for 5 min at 4° to pellet large fragments which were discarded. The supernatant was collected and 15 centrifuged at 40,000 x g for 20 min at 4°. The pellet resulting from this centrifugation was washed once in ice-cold Tris wash buffer and finally resuspended in a final buffer containing 50 mM Tris-HCl and 0.5 mM EDTA, pH 7.4 at 23°. Membrane preparations were kept on ice and utilized within two hours for the radioligand binding assays. Protein concentrations were determined by the 20 method of Bradford (1976) using bovine serum albumin as the standard.

25                   Radioligand Binding: [<sup>3</sup>H]5HT binding was performed using slight modifications of the 5-HT<sub>1D</sub> assay conditions reported by Herrick-Davis and Titeler (1988) with the omission of masking ligands. Radioligand binding studies were achieved at 37° C in a total volume of 250 µl of 30 buffer (50 mM Tris, 10 mM MgCl<sub>2</sub>, 0.2 mM EDTA, 10 µM pargyline, 0.1 % ascorbate, pH 7.4 at 37° C) in 96 well microtiter plates. Saturation studies were conducted using [<sup>3</sup>H]5-HT at 12 different concentrations ranging from 0.5 nM to 100 nM. Displacement studies were 35 performed using 4.5-5.5 nM [<sup>3</sup>H]5-HT. The binding profile of drugs in competition experiments was accomplished using 10-12 concentrations of compound. Incubation times

were 30 min for both saturation and displacement studies based upon initial investigations which determined equilibrium binding conditions. Nonspecific binding was defined in the presence of 10  $\mu$ M 5-HT. Binding was 5 initiated by the addition of 50  $\mu$ l membrane homogenates (10-20  $\mu$ g). The reaction was terminated by rapid filtration through presoaked (0.5% polyethyleneimine) filters using 48R Cell Brandel Harvester (Gaithersburg, MD). Subsequently, filters were washed for 5 sec with ice 10 cold buffer (50 mM Tris HCl, pH 7.4 at 4° C), dried and placed into vials containing 2.5 ml of Readi-Safe (Beckman, Fullerton, CA) and radioactivity was measured using a Beckman LS 5000TA liquid scintillation counter. The efficiency of counting of [ $^3$ H]5HT averaged between 15 45-50%. Binding data was analyzed by computer-assisted nonlinear regression analysis (Accufit and Accucomp, Lndon Software, Chagrin Falls, OH). IC<sub>50</sub> values were converted to K<sub>i</sub> values using the Cheng-Prusoff equation (1973). All experiments were performed in triplicate.

20

#### Measurement of cAMP Formation

Transfected NIH3T3 cells (estimated Bmax from one point competition studies = 488 fmol/mg of protein) were 25 incubated in DMEM, 5mM theophylline, 10mM Hepes (4-[2-Hydroxyethyl]-1-piperazineethanesulfonic acid), 10 $\mu$ M pargyline, for 20 minutes at 37° C, 5% CO<sub>2</sub>. Drug dose-effect curves were then conducted by adding 6 different 30 final concentrations of drug, followed immediately by the addition of forskolin (10  $\mu$ M). Subsequently, the cells were incubated for an additional 10 minutes at 37° C, 5% CO<sub>2</sub>. The media was aspirated and the reaction terminated 35 by the addition of 100 mM HCl. The plates were stored at 4° C for 15 minutes and centrifuged for 5 minutes (500 x g at 4° C) to pellet cellular debris. Aliquots of the supernatant fraction were then stored at -20° C prior to assessment of cAMP formation by radioimmunoassay (cAMP

Radioimmunoassay kit, Advanced Magnetics, Cambridge, MA).

**Tissue Localization Studies.** Human tissues (NDRI) were homogenized and total RNA extracted (Sambrook et al., 1989). cDNA was prepared from 5  $\mu$ g of total RNA with random hexanucleotide primers (500 pmoles) using Superscript reverse transcriptase (BRL) in PCR reaction buffer (Cetus Corp.) containing 1mM dNTPs, at 42°C. for 1 hr. An aliquot of the first strand cDNA was diluted (1:5) in a 50  $\mu$ l PCR reaction mixture (200  $\mu$ M dNTPs final concentration) containing 1.25 U of Taq polymerase and 1  $\mu$ M of primers from the sense strand (5'TCTATTCTGGAGGCACCAAGGAAC3') and from the antisense strand (5'TGTTGATGGGTCAAGATAAGACTT3'). The PCR products were run on a 1.5% agarose gel and transferred to charged nylon membrane (ZetaProbe, Bio-Rad). Filters were hybridized and washed under high stringency.

**In situ Hybridization.** In situ hybridization was performed as described previously (McCabe et al., 1989) using male Hartley guinea pigs (300-350g). A fragment of the guinea pig 5-HT<sub>1F</sub> receptor gene was cloned by homology and sequenced. 45-base oligoprobes synthesized to the 4,5 loop and 5' untranslated regions were 3' end-labeled with <sup>35</sup>S-dATP to a specific activity of  $4 \times 10^9$  Ci/mmol. The nucleotide sequences were: 5'GTGATGCTTGATGCACTCATCATCTCGGCTTGTCCCCCTGGTG 3' and 5'TAGCAGTTCCCTTGAGGTCAAGTTTGATCAGAAGAGTTAAGAA 3'. Sense probes, melting temperature, and RNase pretreatment were used as controls. Sections were exposed to Kodak X-OMAT AR film for 1 week or coated with Kodak NTB-2 emulsion/2% glycerol(1:1) for 2 weeks. Similar experiments were also done on human tissue.

**Drugs:** [<sup>3</sup>H]5-HT (specific activity = 28 Ci/mmol) was obtained from New England Nuclear, Boston, MA. All other chemicals were obtained from commercial sources and

were of the highest grade known purity available.

ResultsCloning of a Novel Gene Encoding a 5HT<sub>1F</sub> Receptor

5 Polyadenylated (poly A+) RNA prepared from rat brain was  
reverse transcribed and the resulting cDNAs were  
subjected to amplification by PCR with the use of a set  
of "degenerate" primers. The synthesis of these primers  
were based on sequences corresponding to the third and  
10 fifth transmembrane segments of the current set of  
available serotonin receptors. The primers were designed  
to amplify only serotonin specific sequences. This was  
accomplished, particularly with the transmembrane domain  
15 V primer, which was designed to anneal at its 3' end only  
to the sequence "AFY(F)IP". We have determined by  
sequence analysis that the presence of an alanine (A)  
rather than a serine (S) in the position immediately  
amino-terminal to the sequence "FY(F)IP" is an amino acid  
20 which can distinguish the closely related adrenergic and  
dopaminergic receptor families from the serotonergic  
receptor family. After 30 amplification cycles, agarose  
gel electrophoresis revealed a clear pattern of cDNA  
species of approximately 250 base pairs. Individual  
25 cDNAs were cloned directly into pBluescript and subjected  
to sequence analysis. One clone, designated S51, was  
observed to encode a novel serotonin receptor. We then  
screened a human genomic placental library with the PCR  
fragment S51. Isolation of the full-length coding region  
30 was obtained from a genomic clone designated h116a.

Nucleotide Sequence and Deduced Amino Acid Sequence of  
h116a

35 DNA sequence information obtained from clone h116a is  
shown in Figure 1. An open reading frame extending from  
an ATG start codon at position 1 to a stop codon at

position 1098 can encode a protein 366 amino acids in length, having a relative molecular mass ( $M_r$ ) of 41,660. A comparison of this protein sequence with previously characterized neurotransmitter receptors indicates that h116a encodes a receptor which is a new member of a family of molecules which span the lipid bilayer seven times and couple to guanine nucleotide regulatory proteins (the G protein-coupled receptor family). A variety of structural features which are invariant in this family were present including the aspartic acid residues of transmembrane regions II and III, the DRY sequence at the end of transmembrane region III, and the conserved proline residues of transmembrane regions IV, V, VI and VII (Hartig et al. and references therein), were present in clone h116a. A comparison of the transmembrane homology of h116a to the other cloned serotonin receptors is shown if Figure 2 exhibits the following order of identity: 5-HT<sub>1D $\alpha$</sub>  (61%), 5-HT<sub>1D $\beta$</sub>  (59%), 5-HT<sub>1A</sub> (54%), 5-HT<sub>1C</sub> (44%) and 5-HT<sub>2</sub> (44%).

20 Receptor Expression in Transfected Mammalian Cells

Saturation analysis of membranes prepared from stably transfected Ltk- cells demonstrated that the receptor expressed was saturable and of high affinity. Scatchard plot analysis by non-linear regression revealed a  $K_d$  of  $9.2 \pm 0.99$  nM (mean  $\pm$  S.E.M., n=4) and a  $B_{max}$   $4.4 \pm 0.36$  picomoles / mg of protein (mean  $\pm$  S.E.M., n=4). The percent specific binding determined at the measured  $K_d$  value for [<sup>3</sup>H]5-HT was greater than 85% of total binding. Furthermore, evidence that the receptor is coupled to a G-protein was demonstrated by the ability of Gpp(NH)p, a non-hydrolyzable analog of GTP, to inhibit the specific binding of [<sup>3</sup>H]5-HT ( $I_{C50} = 243 \pm 115$ ,  $n_H = 0.71 \pm 0.08$ ,  $I_{max} = 55.6 \pm 3.2\%$ ; mean  $\pm$  S.E.M., n=3). Additional data demonstrating that this coupling to a G-protein is functionally relevant is provided below.

5 Pharmacological analysis of the receptor was accomplished by testing the ability of drugs from different chemical classes to displace [<sup>3</sup>H]5-HT specific binding (Table 1).  
10 Of the compounds investigated, 5-HT possessed the highest affinity which according to the classification system of Peroutka and Snyder (1979) makes this site a member of the 5-HT<sub>1</sub> class. Interestingly, 5-CT possessed low affinity and, thus, discriminates this receptor from that of the 5-HT<sub>1D</sub> receptor as well as other members of this class. The one exception appears to be the recently cloned 5-HT<sub>1F</sub> receptor which also has low affinity for 5-  
15 CT (U.S. Serial No. 803,626, filed December 2, 1991, copending). Various ergoline compounds also bound with high affinity including methylergonovine and methysergide. Excluding 1-naphthylpiperazine (Ki = 54), piperazine derivatives had low affinity. Interestingly, the rauwolfia alkaloids, rauwolscine and yohimbine, which are alpha-2 adrenergic antagonists had fair affinity for  
20 this serotonergic receptor. Furthermore, miscellaneous serotonergic agents that possess high affinity for various receptors within the serotonin family including various ketanserin (5-HT<sub>2</sub>), 8-OH-DPAT (5-HT<sub>1A</sub>), DOI (5-HT<sub>1C</sub>/5-  
25 HT<sub>2</sub>), spiperone (5-HT<sub>1A</sub>/5-HT<sub>2</sub>), pindolol (5-HT<sub>1A</sub>/5-HT<sub>1B</sub>) and zacopride (5-HT<sub>3</sub>) had very poor affinity. Taken together, the pharmacological profile of the 5-HT<sub>1F</sub> receptor is unique and contrasts to that of other known serotonin receptors. Interestingly, the agonist rank order of potency (but not antagonist profile) matches one described for large motoneurons in the spinal cord evaluated electrophysiologically (Connel et al., 1989).  
30 Accordingly, the probability of developing selective drugs for this receptor subtype is increased. The functional 5-HT response (1  $\mu$ M) was completely blocked by the nonselective antagonist methiothepin (10  $\mu$ M). This antagonism was surmountable (Fig. 3), indicating probable competitive antagonism. The dose shift produced by  
35

methiothepin yielded an apparent  $K_b$  of  $438 \pm 14$  nM, consistent with the  $K_i$  for this compound (Table 1). No direct effect of methiothepin was observed. No other compound tested in this study was an antagonist. In addition, no evidence for coupling of this receptor to PI turnover was detected at a dose of  $10 \mu\text{M}$  5-HT.

5 -Table 1.  $K_i$  (nM) values of various drugs for the inhibition of [ $^3$ H]5-HT specific binding to clonal 5-HT<sub>1F</sub> cell membranes. Binding assays were performed with 4.5- 5.5 nM of [ $^3$ H]5-HT and 10-12 different concentrations of each inhibitory drug.  $K_i$  values were calculated from the  $IC_{50}$  values using the Cheng-Prusoff equation. Each value is the mean  $\pm$  S.E.M. of 2-4 independent determinations.

	COMPOUND	$K_i$ (nM)
10	5-HT	10.3 $\pm$ 2.0
	Sumatriptan	23.0 $\pm$ 11.0
	Ergonovine	31.0 $\pm$ 1.5
	Methylergonovine	31.0 $\pm$ 11.0
	Methysergide	34.0 $\pm$ 4.9
15	5-Methoxy-N,N-DMT	37.5 $\pm$ 1.5
	1-Naphthylpiperazine	54.0 $\pm$ 3.8
	Yohimbine	92.0 $\pm$ 11.0
	Ergotamine	171 $\pm$ 28
	$\alpha$ -Methyl-5-HT	184 $\pm$ 35
20	NAN 190	203 $\pm$ 13
	Dihydroergotamine	276 $\pm$ 49
	Metergoline	341 $\pm$ 71
	2-Methyl-5-HT	413 $\pm$ 5.6
	Methiothepin	652 $\pm$ 41
25	5-CT	717 $\pm$ 71
	TFMPP	1,002 $\pm$ 85
	S-MT	1,166 $\pm$ 197
	SCH 23390	1,492 $\pm$ 165
	5-Benzoxotryptamine	1,495 $\pm$ 893
30	DP-5-CT	1,613 $\pm$ 817
	DOI	1,739 $\pm$ 84
	8-OH-DPAT	1,772 $\pm$ 38
	5-Fluorotryptamine	1,805 $\pm$ 220
	mCPP	2,020 $\pm$ 36
35	Tryptamine	2,409 $\pm$ 103

5

10

Quipazine	4,668 ± 814
Ritanserin	3,521 ± 86
Propanolol	8,706 ± 97
Ketanserin	>10,000
Spiperone	>10,000
Zacopride	>10,000
Pindolol	>10,000
Mesulergine	>10,000
Harmaline	>10,000
Melatonin	>10,000

cAMP Assay

Additional supporting evidence that the 5-HT<sub>1F</sub> receptor is functionally coupled to a G-protein was obtained by testing the ability of 5-HT as well as other representative serotonergic drugs to inhibit forskolin stimulated cAMP production in NIH3T3 cells transfected with the 5-HT<sub>1F</sub> receptor. The endogenous indoleamine, 5-HT, produced a concentration-related decrease in forskolin-stimulated cAMP production with an EC<sub>50</sub> of 7.1 ± 1.3 nM (n = 4). The maximum inhibition of cAMP production by 5-HT was 67 ± 5.4 %. Additionally, the serotonergic compounds 1-naphthylpiperazine and lysergol inhibited forskolin-stimulated cAMP production with EC<sub>50</sub> values of 4.5 ± 0.2 nM and 8.8 ± 4.3 nM (n = 2), respectively.

Receptor Localization Studies

Expression of the 5-HT<sub>1F</sub> transcripts was analyzed from PCR-northern blots and *in situ* hybridization studies. By PCR, we detected 5-HT<sub>1F</sub> receptor mRNA in the human brain, uterus (endometrium and myometrium) and mesentery (Fig. 4) but not in kidney, liver, spleen, heart, pancreas, or testes. In *in situ* hybridization experiments, we observed 5-HT<sub>1F</sub> transcripts in lamina V of frontal cortex (Fig 5A) in large pyramidal cells (Fig. 5D). Moderate labeling was also detected over layer VI non-pyramidal neurons. In both layer V and layer VI, the labeling was most evident in dorsal sensorimotor neocortex, and in cingulate and retrosplenial cortices (Fig. 5C) . The pyramidal cells in the piriform cortex were heavily labeled as were large neurons in the raphe nuclei (Fig. 5E). Hippocampal pyramidal cells in CA1-CA3 were moderately labeled, as were the granule cells in the dentate gyrus, and some neurons in the nucleus of the solitary tract. Little labeling was found in the

thalamus and hypothalamus. Significant labelling was also found in the large motoneurons of the ventral horn of the spinal cord. The localization in the human was found to be in good concordance with that observed in the  
5 guinea pig.

discussion

5        The deduced amino acid sequence of h116a was analyzed to uncover relationships between it and the other cloned serotonin receptor sequences. Although the homology within the membrane spanning domains was greatest with the 5-HT<sub>1Da</sub> receptor (Fig. 2), the nature of this newly cloned receptor could not be clearly predicted. The rational for this ambiguity is the interpretation of the 10      transmembrane domain homology (approximately 60%) to the 5-HT<sub>1Da</sub> and 5-HT<sub>1Db</sub> receptor subfamily. Closely related members of a "subfamily" of serotonin receptors (i.e. 15      "subtypes") generally share a common transmitter and also have similar pharmacological profiles and physiological roles (for example, 5-HT<sub>2</sub> and 5-HT<sub>1C</sub> or 5-HT<sub>1Da</sub> and 5-HT<sub>1Db</sub>). Such "subtypes" display an amino acid identity of approximately 75-80% in their transmembrane domains. 20      Serotonin receptors which are not members of the same "subfamily", but are members of the serotonin "family" (in which the receptors use the same neurotransmitter; i.e. 5-HT<sub>2</sub> and 5-HT<sub>1Da</sub>) generally show much lower transmembrane homology (approximately 45%). Such 25      transmembrane amino acid homologies can, therefore, give insight into the relationship between receptors and be used as predictors of receptor pharmacology. According to this type of analysis, although the newly cloned receptor appears to be more related to the 5-HT<sub>1D</sub> subfamily, it is likely to be in a subfamily distinct from all the other serotonin receptors. Interestingly, 30      the transmembrane homology between the 5HT<sub>1E</sub> (Levy et al., 1992; McAllister et al., 1992; Zgombick et al., 1992) and 5-HT<sub>1F</sub> (Amlaiky et al., 1992; Adham et al., in press) receptors is 72%. It is therefore possible that these 35      receptors may be "subtypes", rather than members of distinct "subfamilies".

The present pharmacological evidence substantiates the existence of a novel serotonin receptor in the human brain and peripheral tissues. Comparison of the binding affinities for various drugs observed in native membranes 5 for other known serotonergic receptors (see Hoyer, 1989) to that of the 5-HT<sub>1F</sub> receptor demonstrates that the pharmacological profile does not fit any known receptor to date. The cloning of the 5-HT<sub>1F</sub> site will now allow more extensive investigations into the nature of this 10 unique serotonergic receptor.

The structure-activity relationships observed in the present study suggest that there are important requirements for high affinity binding to the 5-HT<sub>1F</sub> receptor. Substitution or removal of the 5-hydroxy group 15 on serotonin significantly decreases the affinity for the receptor (e.g., tryptamine, 5-methoxytryptamine and 5-carboxyamidotryptamine). Additionally,  $\alpha$ -methylation and 2-methylation of 5-HT lowers its affinity by 20 and 40 fold, respectively, for the 5-HT<sub>1F</sub> site. In contrast to 20 these substitutions, N,N-dimethylation of the aliphatic side chain of the indole ring increases the affinity approximately 20 fold (unpublished observations). Interestingly, 5-methoxy-N,N-dimethyltryptamine which 25 possesses both a 5-hydroxy substitution as well as a N,N-dimethylation has an affinity much higher than the other 5-substituted tryptamine derivatives. Basic structural requirements of the ergoline derivatives demonstrate that 30 N-methylation of the indole ring does not decrease affinity as does bulky substitutions. Furthermore, piperazine derivatives are not bound at high affinity.

Notably, the application of the human 5-HT<sub>1F</sub> receptor 35 clone to pharmaceutical research can lead to new drug design and development. In this regard, it is important to point out that the affinities of sumatriptan, methylergonovine and methysergide for this receptor

suggest that this site may be involved in the control of migraine headaches. Certainly, these compounds have had success in the clinic for the treatment of this debilitating disorder (Sleight et al., 1990). Notably, however, it has been thought that the action of these compounds is mediated at 5-HT<sub>1D</sub> receptors for sumatriptan and 5-HT<sub>2</sub> receptors for methysergide. Interestingly, methylergonovine may be an active metabolite of methysergide which can be responsible for some of the therapeutic antimigraine effects of methysergide. This novel site with affinity for these agents would now suggest that there is one serotonergic receptor which may be responsible for both the pathogenesis and, accordingly, the pharmacological treatment. Importantly, the agents prescribed for migraine are not selective for any one particular serotonin receptor and, thus, the physiological significance of drugs acting at one specific site remains controversial (Humphrey P.P.A. et al., 1990). The notion that the 5-HT<sub>1F</sub> receptor is involved in migraine may be supported by evidence demonstrating that metergoline which has high affinity for the 5-HT<sub>1D</sub> receptor does not block the effects of sumatriptan in the dog saphenous vein (Sumner and Humphrey, 1990) inferring that this vascular model may contain the novel 5-HT<sub>1F</sub> site. Furthermore, this data can support the idea that sumatriptan acts at 5-HT<sub>1F</sub> receptors as an anti-migraine drug. Localization of transcripts for the 5-HT<sub>1F</sub> receptor in the spinal trigeminal nucleus by *in situ* hybridization strongly supports this contention (Buzzi et al., 1990, 1991; Moskowitz et al., 1992). The potential of the 5-HT<sub>1F</sub> receptor as a novel target for migraine where selective drugs may be developed is an exciting possibility which needs to be explored.

35

Further insight into potential therapeutic significance of the 5-HT<sub>1F</sub> receptor has been obtained through

localization studies using PCR and *in situ* hybridization. Localization of transcripts for this receptor indicates a relatively selective tissue distribution. Of tissues reported here, the 5-HT<sub>1F</sub> receptor was only detected in 5 a few including the brain, uterus, and mesentery. The possible role of this receptor in uterine or vascular function is intriguing. Future studies defining the specific cell type(s) in these tissues which express the receptor may provide insight into its function in the 10 periphery. Possibilities for therapeutic benefit include dysmenorrhea and labor induction uterus) and hypertension (vascular components of mesentery) and obesity (adipose components). In the brain, the expression of the 5-HT<sub>1F</sub> receptor had a limited distribution compared to that of other serotonin receptors . In the neocortex, labelling 15 of layer V pyramidal neurons may indicate a functional role for the 5-HT<sub>1F</sub> receptor protein in the integration of sensorimotor (somatodendritic; frontal cortex) or afferent information associated with limbic functions (somatodendritic; cingulate/retrosplenial cortex), or in 20 spinal cord processes (axonal). Intense labeling was detected in the large motoneurons of the ventral horn of the spinal cord. Strong labeling was also detected in hippocampal pyramidal cells, in several thalamic nuclei, 25 and in the dorsal raphe. The detection of transcripts for this gene in the dorsal raphe nucleus indicates a possible role as an autoreceptor. Autoreceptor function opens the possibility that the 5-HT<sub>1F</sub> receptor could be involved in any or all of the known actions of serotonin 30 including therapeutic potential in anxiety, depression, sleep disorders including jet lag, appetite control, sexual dysfunction, gastrointestinal motility including irritable bowel disease, and cardiovascular regulation. In addition, localization to the large motoneurons 35 indicates a possible role in spasticity and other disorders of movement.

Another consideration for therapeutic application of this site may be related to the treatment of feeding disorders such as obesity, bulimia nervosa and/or anorexia nervosa. The involvement of serotonin and feeding behavior has received much attention during the last decade. It is now known that many of the identified and well-characterized serotonergic receptors are capable of modulating feeding (Blundell and Lawton, 1990). Notably, serotonin uptake blockers which have been used to treat feeding disorders act nonselectively and as such have side-effect potential (Jimerson et al., 1990). The fact that the 5-HT<sub>1F</sub> receptor has been cloned from both peripheral and central sites, and has been localized by both PCR and by in situ hybridization, suggests from an anatomical standpoint that it can be found in strategic locations where feeding may be altered. Although many different serotonergic receptors are involved in feeding, the search for the one site that can be exploited for selective drug development has yet to be found. There is no doubt that interest exists in finding drugs that interact with the serotonin system for the treatment of feeding disorders (Cooper, 1989).

Overall, the 5-HT<sub>1F</sub> receptor can be an important site stimulated by nonselectively blocking serotonin uptake as is accomplished with certain antidepressants. In regard to this, serotonin uptake blockers are effective in treating neuropsychiatric disorders such as depression and obsessive-compulsive illness (Asberg et al., 1986; Sleight et al., 1990; Insel et al., 1985). However, these agents have side effects and, in fact, the mechanism of action for these compounds are not linked to any particular serotonergic receptor. The possibility that agents selective for the 5-HT<sub>1F</sub> receptor may have clinical utility as antidepressants, for example, without the side effects attributed to current treatment modalities can have significant implications for drug

therapy. The localization of the 5-HT<sub>1F</sub> receptor in the raphe nuclei, and therefore its potential role as an autoreceptor, further supports the role for this receptor subtype in depression.

5 In summary, the pharmacological profile of the cloned human 5-HT<sub>1F</sub> receptor is unique and contrasts to other known serotonergic receptors. The utility of this site expressed in a cellular system and, thus, isolated for 10 study will create excellent opportunities in drug development directed towards a novel serotonergic receptor that may have wide-range implications for drug therapy. Ultimately, indepth investigations into the 15 localization of this receptor in brain and peripheral tissue will target new sites that may lead to functional roles of the serotonergic receptor. Indeed, the potential therapeutic applications may extend to neuropsychiatric disorders including depression, anxiety, schizophrenia, dementia and obsessive-compulsive illness as well as 20 obesity and migraine.

Additionally, the localization of the 5-HT<sub>1F</sub> receptor in the spinal cord suggests possible roles for this subtype in analgesia as well as spasticity. The clear evidence 25 of involvement of this receptor in the ventral horn further supports the possible role in motor control. Interestingly, the agonist profile of the 5-HT<sub>1F</sub> receptor matches that reported for large motoneurons of the spinal 30 cord measured electrophysiologically (Connel et al., 1989). In addition, the presence of the 5-HT<sub>1F</sub> receptor in the mesentery, at major resistance bed of the vascular tree, indicated a role in the control of blood pressure. A detailed accounting of the localization and therapeutic potential is presented in Table II.

-59-

Table II. Summary of the localization of mRNA for the 5-HT<sub>1F</sub> receptor in the guinea pig and human CNS. Experiments were performed as described (methods). Each experiment was replicated 2-3 times. Potential therapeutic roles anticipated base on these data are indicated.

-60-

LOCALIZATION OF HUMAN 5HT<sub>1F</sub> mRNA\*

AREAS	PROJECTIONS	THERAPEUTIC RELEVANCE
FRONTAL CORTEX	Main projections to striatum, dorsal thalamus, and superior colliculus.	Potential application for the development of treatments for schizophrenia and mood disorders.
CAUDATE NUCLEUS	Primary projections to globus pallidus, substantia nigra.	Potential treatment of any basal ganglia disorder, including Parkinson's disease, Huntington's chorea, or tardive dyskinesia.
HIPPOCAMPAL FORMATION	Pyramidal neurons project mainly within the hippocampus, and also to the septum.	Primary locus for treatment of memory disorders, e.g. Alzheimer's disease or for cognitive enhancement in people with learning disabilities. Also possible treatment for temporal lobe epilepsy.
AMYGDALA	Cells in amygdala have widespread projections to cortex, hippocampus, basal ganglia, hypothalamus, and brainstem autonomic centers.	Wide range of potential applications. These include treatment of autonomic dysfunctions such as cardiac arrhythmias and non-adaptive response to environmental stressors. Also potential treatment of mood disorders, such as bipolar syndrome.

Table 2 continued

-61-

AREAS	PROJECTIONS	THERAPEUTIC RELEVANCE
HYPOGLOSSAL NUCLEUS	Main projections to somatic skeletal musculature of the tongue.	Treatment of verbal apraxia.
DORSAL EFFERENT NUCLEUS OF THE VAGUS	Principal projections are to the parasympathetic ganglia and abdominal viscera.	May have some application to the treatment of stress-related ulcers and irritable bowel disease.
NUCLEUS OF THE SOLITARY TRACT	Main projections are to thalamus, amygdala, rostroventral medulla, and the A1 noradrenergic cell group of the dorsal medulla.	Varied potential applications, with regulation of cardiovascular function the most prominent, e.g. an anti-hypertensive.
GRACILE NUCLEUS	Provides innervation of lumbosacral spinal cord.	Potential applications for the treatment of dermatitis, or pain associated with itching.
CUNEATE NUCLEUS	Provides innervation of cervical spinal cord.	Potential applications for the treatment of dermatitis, or pain associated with itching.

Table 2 continued

-62-

AREAS	PROJECTIONS	THERAPEUTIC RELEVANCE
SPINAL TRIGEMINAL NUCLEUS	Main projections are to the contralateral ventrobasal thalamus, the posterior thalamic n., the zona incerta, the superior colliculus, and the motor nuclei of trigeminal.	Potential treatment of migraine headaches, and other pain syndromes such as trigeminal neuralgia.
OLIVARY COMPLEX	Primary projections are to the cerebellum.	Treatment of ataxia associated with olivopontocerebellar atrophy, or tremors accompanying some neurodegenerative diseases
RETICULAR FORMATION	Projections to the intra-laminar and dorsomedial n. of thalamus, the hypothalamus, supramammillary and lateral mammillary nuclei, the septum, the diagonal band, spinal cord, cerebellum, brainstem autonomic nuclei.	Involvement in cardiac pressor and depressor responses suggests a role in blood pressure regulation and possibly a treatment for hypertension. Also possible application for the treatment of urinary retention disorders, and in the management of pain.
MEDIAL VESTIBULAR NUCLEUS	Projections to oculomotor complex and cervical spinal cord motor neurons.	Treatment of motion sickness.

Table 2 continued

AREAS	PROJECTIONS	THERAPEUTIC RELEVANCE
CEREBELLAR PURKINJE CELLS	Projections only to deep cerebellar nuclei.	Potential treatment of movement disorders, particularly those involving planned movements, or those involving abnormalities of gait or stance.
SPINAL CORD VENTRAL HORN	Ascending dorsal horn projections to thalamus, brainstem reticular formation and central gray. Ventral horn projections to skeletal and/or smooth muscle.	Primary site for treatment of pain, and for possible anesthetic applications. Also possible therapies for spasticity and movement disorders.

Table 2 continued

-64-

LOCALIZATION OF GUINEA PIG SHT<sub>1</sub>, ERNA

AREAS	PROJECTIONS	RELEVANCE TO HUMAN THERAPIES
ANTERIOR OLFACTORY NUCLEI and PIRIFORM CORTEX	Widespread projections to brain olfactory centers, to limbic system, hypothalamus, thalamus, and striatum.	Treatment of olfactory disorders (dysosmias) associated with many neurological syndromes.
LAYER V of NEOCORTEX	Cells of layer V project primarily to other cortical areas, and to basal ganglia.	Enhancement of memory for motor tasks, particularly in certain amnestic syndromes, e.g. Alzheimer's disease.
CAUDATE-PUTAMEN and NUCLEUS ACCUMBENS	Medium spiny neurons project to globus pallidus, entopeduncular n., and substantia nigra.	Potential treatment of any basal ganglia disorder, including Parkinson's disease, Huntington's chorea, or tardive dyskinesia.
AMYGDALA	Cells in amygdala have widespread projections to cortex, hippocampus, basal ganglia, hypothalamus, and brainstem autonomic centers.	Wide range of potential applications. These include treatment of autonomic dysfunctions such as cardiac arrhythmias and non-adaptive response to environmental stressors. Also potential treatment of mood disorders, such as bipolar syndrome.

Table 2 continued

-65-

AREAS	PROJECTIONS	RELEVANCE TO HUMAN THERAPIES
HIPPOCAMPUS	Pyramidal neurons project mainly within the hippocampus, and also to the septum.	Primary locus for treatment of memory disorders, e.g. Alzheimer's disease or for cognitive enhancement in people with learning disabilities. Also possible treatment for temporal lobe epilepsy.
DORSAL RAPHE	Extensive projections to cerebral cortex, frontal striatum, limbic structures, olfactory tubercle, central gray, hippocampus, and spinal cord.	Treatment of pain syndromes, including migraine headache. Involvement of raphe in general arousal/attentional processes makes this a possible target for treatment of attentional dysfunctions, such as those observed in Alzheimer's disease, or in developmental disabilities. Potential application in the treatment of depression.
PONTINE NUCLEI	Major projection is to the cerebellar cortex.	Potential treatment of movement disorders, particularly planned movement, and gait disorders such as Friedrich's ataxia.
INFERIOR COLICULUS	Major obligatory synaptic station in ascending auditory pathway.	

Table 2 continued

-66-

AREAS	PROJECTIONS	RELEVANCE TO HUMAN THERAPIES
TRIGEMINAL NUCLEAR COMPLEX	Main projections are to the contralateral ventrobasal thalamus, the posterior thalamic n., the zona incerta, the superior colliculus, and the motor nuclei of trigeminal.	Potential treatment of migraine headaches, and other pain syndromes such as trigeminal neuralgia.
PONTINE RETICULAR FORMATION A. GIGANTOCELLULAR RETICULAR NUCLEUS B. PARAGIGANTOCELLULAR RETICULAR NUCLEUS C. RAPHE MAGNUS	Projections to the intra-laminar and dorsomedial n. of thalamus, the hypothalamus, supramammillary and lateral mammillary nuclei, the septum, the diagonal band, spinal cord, cerebellum, brainstem autonomic nuclei	Involvement in cardiac pressor and depressor responses suggests a role in blood pressure regulation and possibly a treatment for hypertension. Also possible application for the treatment of urinary retention disorders, and in the management of pain.
MEDIAL VESTIBULAR NUCLEUS	Projections to oculomotor complex and cervical spinal cord motor neurons.	Treatment of motion sickness.
CEREBELLAR PURKINJE CELLS	Projections only to deep cerebellar nuclei.	Potential treatment of movement disorders, particularly those involving planned movements, or those involving abnormalities of gait or stance.

Table 2 continued

-67-

SPINAL CORD	Ascending dorsal horn projections to thalamus, brainstem reticular formation and central gray. Ventral horn projections to skeletal and/or smooth muscle.	Primary site for treatment of pain, and for possible anesthetic applications. Also possible therapies for spasticity and movement disorders.
-------------	---	--

### References

5 Adham, N., Kao, H-T., Schechter, L.E., Bard, J., Olsen, M., Urquhart, D., Durkin, M., Hartig, P.R., Weinshank R.L., and Branchek, T.A. Cloning of Another Human Serotonin Receptor (5-HT<sub>1F</sub>): A fifth 5-HT<sub>1</sub> Receptor Subtype Coupled to the Inhibition of Adenylyl Cyclase. Proc. Natl. Acad. Sci. U.S.A., in press.

10 Amlaiky, N., Ramboz, S., Boschert, U., Plassat, J-L. and Hen, R.: Isolation of a mouse "5HT1E-like" serotonin receptor expressed predominantly in hippocampus. J. Biol. Chem. 267:19761-19764, 1992.

15 Asberg, M., Eriksson, B., Matensson, B., Traskman-Bendz, L. and Wagner, A.: Therapeutic effects of serotonin uptake inhibitors in depression. J. Clin. Psychiat. 47:23-35, 1986.

20 Blundell, J.E. and Lawton, C.L.: Serotonin receptor subtypes and the organisation of feeding behaviour: Experimental models. In: Serotonin: From cell biology to pharmacology and therapeutics. (eds. Paoletti, R., Vanhoutte, P.M., Brunello, N. and Maggi, F.M.) Boston:Kluwer Academic Publishers, pp 213-219, 1990.

25 Bradford, M.: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254, 1976.

30 Branchek, T., Weinshank, R.L., Macchi, M.J., Zgombick, J.M. and Hartig, P.R.: Cloning and expression of a human 5-HT1D receptor. The Second IUPHAR Satellite Meeting on Serotonin, Basel, Switzerland, July 11-13, 1990, Abstract # 2.

- 69 -

Buzzi, M.G. and Moskowitz, M.A.: the antimigraine drug, sumatriptan (GR43175), selectively blocks neurogenic plasma extravasation from blood vessels in dura mater. Br. J. Pharmacol. 99:202-206, 1990.

5 Buzzi, M.G., Moskowitz, M.A., Peroutka, S.J. and Byun,  
B.: Further characterization of the putative 5-HT  
receptor which mediates blockade of neurogenic plasma  
extravasation in rat dura mater. Br. J.Pharmacol.  
0 103:1421-1428, 1991.

Cheng, Y.C. and Prusoff, W.H.: Relationship between the inhibition constant ( $K_i$ ) and the concentration of inhibitor which causes 50% inhibition ( $IC_{50}$ ) of an enzyme reaction. *Biochem. Pharmacol.* 22:3099-3108, 1973.

Connel, L.A. and Wallis, D.I.: 5-hydroxytryptamine depolarizes neonatal rat motorneurones through a receptor unrelated to an identified binding site. *Neuropharmacology* 28:625-634, 1989.

Cooper, S.J.: Drugs interacting with 5-HT systems show promise for treatment of eating disorders. TIPS 10:56-57, 1989.

25 Fargin, A., Raymond, J.R., Lohse, M.J., Kobilka, B.K.  
Caron, M.G. and Lefkowitz, R.J.: The genomic clone G-21  
which resembles a  $\beta$ -adrenergic receptor sequence encodes  
the 5-HT<sub>1A</sub> receptor. *Nature* 335:358-360, 1988.

30 Feinberg, A.P., and Vogelstein, B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal.Biochem.* 132:6-13, 1983.

35 Gaddum, J.H. and Picarelli, Z.P.: Two kinds of tryptamine receptor. Brit. J. Pharmacol. 12:323-328, 1957.

-70-

Glennon, R.A.: Serotonin receptors: Clinical implications. *Neurosci. Biobehav. Rev.* 14:35-47, 1990.

5 Green, A.R.: *Neuropharmacology of serotonin*. Oxford: Oxford University Press, 1985.

10 Hamon, M., Lanfumey, L., El Mestikawy, S., Boni, C., Miquel, M.-C., Bolanos, F., Schechter, L. and Gozlan, H.: The main features of central 5-HT<sub>1</sub> receptors. *Neuropsychopharmacol.* 3(5/6):349-360, 1990.

15 Hartig, P.R., Kao, H.-T., Macchi, M., Adham, N., Zgombick, J., Weinshank, R. and Branchek, T.: The molecular biology of serotonin receptors: An overview. *Neuropsychopharmacol.* 3(5/6):335-347, 1990.

20 Herrick-Davis K. and Titeler, M.: Detection and characterization of the serotonin 5-HT<sub>1D</sub> receptor in rat and human brain. *J. Neurochem.* 50:1624-1631, 1988.

25 Hoyer, D.: Biochemical mechanisms of 5-HT receptor-effector coupling in peripheral tissues. In: *Peripheral actions of 5-HT*. (ed. Fozard, J.R.) Oxford:Oxford University Press, pp 72-99, 1989.

30 Insel, T.R., Mueller, E.A., Alterman, I., Linnoila, M. and Murphy, D.L.: Obsessive-compulsive disorder and serotonin: Is there a connection? *Biol. Psychiat.* 20:1174-1188, 1985.

35 Jimerson, D.C., Lesem, M.D., Hegg, A.P. and Brewerton, T.D.: Serotonin in human eating disorders. *Ann. N.Y. Acad. Sci.* 600:532-544, 1990.

-71-

Julius, D., MacDermott, A.B., Axel, R. and Jessell, T.M.: Molecular characterization of a functional cDNA encoding the serotonin 1C receptor. *Science* 241:558-564, 1988.

5 Leonhardt, S., Herrick-Davis, K. and Titeler, M.: Detection of a novel serotonin receptor subtype (5-HT1F) in human brain: Interaction with a GTP-binding protein. *J. Neurochem.* 53(2):465-471, 1989.

10 Levy, F. O., Gudermann, T., Birnbaumer, M., Kaumann, A. J., & Birnbaumer, L. Molecular cloning of a human gene (S31) encoding a novel serotonin receptor mediating inhibition of adenylyl cyclase. *FEBS Lett.* 296, 201-206, 1992.

15 Maniatis, T., Fritsch, E.F., and Sambrook, J. *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982.

20 McAllister, G., Charlesworth, A., Snodin, C., Beer, M. S., Noble, A. J., Middlemiss, D. N., Iversen, L. L., & Whiting, P. Molecular cloning of a serotonin receptor from human brain (5-HT1E): A fifth 5HT1-like subtype. *Proc. Natl. Acad. Sci. (USA)*. 89, 5517-5521, 1992.

25 McCabe, J.T. and Pfaff, D.W., in *Gene Probes* (Academic Press, San Diego) Conn, P.M. (ed.) pp. 98-126, 1989.

30 Moskowitz, M.A. Neurogenic versus vascular mechanisms of sumatriptan and ergot alkaloids in migraine. *Trends Pharmacol. Sci.* 13:307-311, 1992.

Osborne, N.N. and Hamon, M.: *Neuronal serotonin*. Chichester: John Wiley and Sons, Inc., 1988.

35 Peroutka, S.J.: *Serotonin receptor subtypes: Basic and clinical aspects*. New York: Wiley-Liss, Inc., 1991.

-72-

Peroutka, S.J. and Snyder, S.H.: Multiple serotonin receptors, differential binding of [<sup>3</sup>H]5-hydroxytryptamine, [<sup>3</sup>H]lysergic acid diethylamide and [<sup>3</sup>H]spiroperidol. *Mol. Pharmacol.* 16:687-699, 1979.

5 Pritchett, D.B., Bach, A.W.J., Wozny, M., Taleb, O., Dal Toso, R., Shih, J. and Seeburg, P.H.: Structure and functional expression of cloned rat serotonin 5-HT<sub>2</sub> receptor. *EMBO J.* 7:4135-4140, 1988.

10 Rapport, M.M., Green, A.A. and Page, I.H.: Purification of the the substance which is responsible for vasoconstrictor activity of serum. *Fed. Proc.* 6:184, 1947.

15 Rapport, M.M.: Serum vasoconstrictor (serotonin) V. Presence of creatinine in the complex. A proposed structure of the vasoconstrictor principle. *J. Biol. Chem.* 180:961-969, 1949.

20 Sambrook, J., Fritsch, E. F., & Maniatis, T., in *Molecular Cloning: A Laboratory Manual*; 2nd edition. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 7.19-7.22, 1989.

25 Sanders-Bush, E.: *The Serotonin Receptors*. Clifton, New Jersey: Humana Press, 1988.

30 Sleight, A.J., Pierce, P.A., Schmidt, A.W., Hekmatpanah, C.R. and Peroutka, S.J.: The clinical utility of serotonin receptor active agents in neuropsychiatric disease. In: *Serotonin receptor subtypes: Basic and clinical aspects*. (ed. Peroutka, S.J.) New York:Wiley-Liss, Inc., pp 211-227, 1990.

35 Sumner, M.J. and Humphrey, P.P.A.: Sumatriptan (GR43175) inhibits cyclic-AMP accumulation in dog isolated

-73-

saphenous vein. Br. J. Pharmacol. 99:219-220, 1990.

5 Zgombick, J.M., Schechter, L.E., Macchi, M., Hartig, P.R., Branchek, T.A., and Weinshank, R.L. The human gene S31 encodes the pharmacologically-defined serotonin 5-HT<sub>1E</sub> receptor. Mol. Pharmacol., 42, 180-185, 1992.

-74-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Weinhank, Richard L.  
Branchek, Theresa  
Hartig, Paul R.

(ii) TITLE OF INVENTION: DNA ENCODING A HUMAN 5-HT1F RECEPTOR AND  
USES THEREOF

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Cooper & Dunham  
(B) STREET: 30 Rockefeller Plaza  
(C) CITY: New York  
(D) STATE: New York  
(E) COUNTRY: USA  
(F) ZIP: 10112

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: White, John P  
(B) REGISTRATION NUMBER: 28,678  
(C) REFERENCE/DOCKET NUMBER: 1795/39318

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 212-977-9550  
(B) TELEFAX: 212-664-0525  
(C) TELEX: 422523 COOP UI

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1730 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: both  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vii) IMMEDIATE SOURCE:

-75-

(A) LIBRARY: human lymphocyte genomic  
 (B) CLONE: h116a

## (ix) FEATURE:

(A) NAME/KEY: CDS  
 (B) LOCATION: 616..1713

## (ix) FEATURE:

(A) NAME/KEY: mat\_peptide  
 (B) LOCATION: 616..1713

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTGCATGCCT GCAGGTCGAC TCTAGAGGAT CCCCCGGTAC CGAGCTCGAA TTCCCTTGTT	60
ATTTTGTCA GCTTCAAGCC TAGGAAAAGC CTAAGCAAAA CTCTTGGTGG GCTCTTGTT	120
ACATTCCAGC CTTTGAATAA GGGCACTGGC TCTATCAGCT TTGAATATAT AACTCAACTA	180
GTCAGTCAGT AGTACTGAAA CAGTTGTTAC GGAGGCCTGC GTTATTGAGA TCGGGCCTGC	240
CACACTTTA AACTTTTCT GACATGGACA AAGAGAAAAAA CCAATTCTAT AATGGCAGAG	300
ATTTCACTGA GTAACAAGCT AGACTATCAT TAAAAATTGT TGTATTAAC CTATATTTA	360
AGAAATGTTT TGGAAAGTTAC TGGCTTTTT TACTGTTCTC ATTAATTTTC TTAAATAAAAA	420
AGGAAAACAA AAACCTTCAA TCTGAACCTC ATTTTTTAA TCTATAGAAT ATTCTGGTA	480
AACATAACAT ACACTTTTA AAAATTATTC TGAAAGGAAG AGAAAAGTTC TTGAACCCCTT	540
CTCTGAAC TG TTTTTCTCT TCCCTTGTAA CAGGTATCCA TTTTCAGCT ATATTAATCT	600
TTTAAACAA AGAAA ATG GAT TTC TTA AAT TCA TCT GAT CAA AAC TTG ACC	651
Met Asp Phe Leu Asn Ser Ser Asp Gln Asn Leu Thr	
1 5 10	
TCA GAG GAA CTG TTA AAC AGA ATG CCA TCC AAA ATT CTG GTG TCC CTC	699
Ser Glu Glu Leu Leu Asn Arg Met Pro Ser Lys Ile Leu Val Ser Leu	
15 20 25	
ACT CTG TCT GGG CTG GCA CTG ATG ACA ACA ACT ATC AAC TCC CTT GTG	747
Thr Leu Ser Gly Leu Ala Leu Met Thr Thr Ile Asn Ser Leu Val	
30 35 40	
ATC GCT GCA ATT ATT GTG ACC CGG AAG CTG CAC CAT CCA GCC AAT TAT	795
Ile Ala Ala Ile Ile Val Thr Arg Lys Leu His His Pro Ala Asn Tyr	
45 50 55 60	
TTA ATT TGT TCC CTT GCA GTC ACA GAT TTT CTT GTG GCT GTC CTG CTG	843
Leu Ile Cys Ser Leu Ala Val Thr Asp Phe Leu Val Ala Val Leu Val	
65 70 75	
ATG CCC TTC AGC ATT GTG TAT ATT GTC AGA GAG AGC TGG ATT ATG GGG	891
Met Pro Phe Ser Ile Val Tyr Ile Val Arg Glu Ser Trp Ile Met Gly	
80 85 90	
CAA GTG GTC TGT GAC ATT TGG CTG AGT GTT GAC ATT ACC TGC TGC ACG	939
Gln Val Val Cys Asp Ile Trp Leu Ser Val Asp Ile Thr Cys Cys Thr	
95 100 105	

-76-

TGC TCC ATC TTG CAT CTC TCA GCT ATA GCT TTG GAT CGG TAT CGA GCA Cys Ser Ile Leu His Leu Ser Ala Ile Ala Leu Asp Arg Tyr Arg Ala 110 115 120	987
ATC ACA GAT GCT GTT GAG TAT GCC AGG AAA AGG ACT CCA AAG CAT GCT Ile Thr Asp Ala Val Glu Tyr Ala Arg Lys Arg Thr Pro Lys His Ala 125 130 135 140	1035
GGC ATT ATG ATT ACA ATA GTT TGG ATT ATA TCT GTT TTT ATC TCT ATG Gly Ile Met Ile Thr Ile Val Trp Ile Ile Ser Val Phe Ile Ser Met 145 150 155	1083
CCT CCT CTA TTC TGG AGG CAC CAA GGA ACT AGC AGA GAT GAT GAA TGC Pro Pro Leu Phe Trp Arg His Gln Gly Thr Ser Arg Asp Asp Glu Cys 160 165 170	1131
ATC ATC AAG CAC GAC CAC ATT GTT TCC ACC ATT TAC TCA ACA TTT GGA Ile Ile Lys His Asp His Ile Val Ser Thr Ile Tyr Ser Thr Phe Gly 175 180 185	1179
GCT TTC TAC ATC CCA CTG GCA TTG ATT TTG ATC CTT TAC TAC AAA ATA Ala Phe Tyr Ile Pro Leu Ala Leu Ile Leu Ile Leu Tyr Tyr Lys Ile 190 195 200	1227
TAT AGA GCA GCA AAG ACA TTA TAC CAC AAG AGA CAA GCA AGT AGG ATT Tyr Arg Ala Ala Lys Thr Leu Tyr His Lys Arg Gln Ala Ser Arg Ile 205 210 215 220	1275
GCA AAG GAG GAG GTG AAT GGC CAA GTC CTT TTG GAG AGT GGT GAG AAA Ala Lys Glu Glu Val Asn Gly Gln Val Leu Leu Glu Ser Gly Glu Lys 225 230 235	1323
AGC ACT AAA TCA GTT TCC ACA TCC TAT GTA CTA GAA AAG TCT TTA TCT Ser Thr Lys Ser Val Ser Thr Ser Tyr Val Leu Glu Lys Ser Leu Ser 240 245 250	1371
GAC CCA TCA ACA GAC TTT GAT AAA ATT CAT AGC ACA GTG AGA AGT CTC Asp Pro Ser Thr Asp Phe Asp Lys Ile His Ser Thr Val Arg Ser Leu 255 260 265	1419
AGC TCT GAA TTC AAG CAT GAG AAA TCT TGG AGA AGG CAA AAG ATC TCA Arg Ser Glu Phe Lys His Glu Lys Ser Trp Arg Arg Gln Lys Ile Ser 270 275 280	1467
GCT ACA AGA GAA CGG AAA GCA GCC ACT ACC CTG GGA TTA ATC TTG GGT Gly Thr Arg Glu Arg Lys Ala Ala Thr Thr Leu Gly Leu Ile Leu Gly 285 290 295 300	1515
GCA TTT GTA ATA TGT TGG CTT CCT TTT GTA AAA GAA TTA GTT GTT Ala Phe Val Ile Cys Trp Leu Pro Phe Phe Val Lys Glu Leu Val Val 305 310 315	1563
AAT GTC TGT GAC AAA TGT AAA ATT TCT GAA GAA ATG TCC AAT TTT TTG Asn Val Cys Asp Lys Cys Lys Ile Ser Glu Glu Met Ser Asn Phe Leu 320 325 330	1611
GCA TGG CTT GGG TAT CTC AAT TCC CTT ATA AAT CCA CTG ATT TAC ACA Ala Trp Leu Gly Tyr Leu Asn Ser Leu Ile Asn Pro Leu Ile Tyr Thr 335 340 345	1659

-77-

ATC TTT AAT GAA GAC TTC AAG AAA GCA TTC CAA AAG CTT GTG CGA TGT 1707  
 Ile Phe Asn Glu Asp Phe Lys Lys Ala Phe Gln Lys Leu Val Arg Cys  
 350 355 360

CGA TGT TAGTTTAAA AATGTTT 1730  
 Arg Cys  
 365

## (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 366 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Asp Phe Leu Asn Ser Ser Asp Gln Asn Leu Thr Ser Glu Glu Leu  
 1 5 10 15

Leu Asn Arg Met Pro Ser Lys Ile Leu Val Ser Leu Thr Leu Ser Gly  
 20 25 30

Leu Ala Leu Met Thr Thr Ile Asn Ser Leu Val Ile Ala Ala Ile  
 35 40 45

Ile Val Thr Arg Lys Leu His His Pro Ala Asn Tyr Leu Ile Cys Ser  
 50 55 60

Leu Ala Val Thr Asp Phe Leu Val Ala Val Leu Val Met Pro Phe Ser  
 65 70 75 80

Ile Val Tyr Ile Val Arg Glu Ser Trp Ile Met Gly Gln Val Val Cys  
 85 90 95

Asp Ile Trp Leu Ser Val Asp Ile Thr Cys Cys Thr Cys Ser Ile Leu  
 100 105 110

His Leu Ser Ala Ile Ala Leu Asp Arg Tyr Arg Ala Ile Thr Asp Ala  
 115 120 125

Val Glu Tyr Ala Arg Lys Arg Thr Pro Lys His Ala Gly Ile Met Ile  
 130 135 140

Thr Ile Val Trp Ile Ile Ser Val Phe Ile Ser Met Pro Pro Leu Phe  
 145 150 155 160

Trp Arg His Gln Gly Thr Ser Arg Asp Asp Glu Cys Ile Ile Lys His  
 165 170 175

Asp His Ile Val Ser Thr Ile Tyr Ser Thr Phe Gly Ala Phe Tyr Ile  
 180 185 190

Pro Leu Ala Leu Ile Leu Ile Tyr Tyr Lys Ile Tyr Arg Ala Ala  
 195 200 205

Lys Thr Leu Tyr His Lys Arg Gln Ala Ser Arg Ile Ala Lys Glu Glu  
 210 215 220

-78-

Val	Asn	Gly	Gln	Val	Leu	Leu	Glu	Ser	Gly	Glu	Lys	Ser	Thr	Lys	Ser
225							230				235				240
Val	Ser	Thr	Ser	Tyr	Val	Leu	Glu	Lys	Ser	Leu	Ser	Asp	Pro	Ser	Thr
							245				250				255
Asp	Phe	Asp	Lys	Ile	His	Ser	Thr	Val	Arg	Ser	Leu	Arg	Ser	Glu	Phe
							260			265				270	
Lys	His	Glu	Lys	Ser	Trp	Arg	Arg	Gln	Lys	Ile	Ser	Gly	Thr	Arg	Glu
							275			280				285	
Arg	Lys	Ala	Ala	Thr	Thr	Leu	Gly	Leu	Ile	Leu	Gly	Ala	Phe	Val	Ile
							290			295				300	
Cys	Trp	Leu	Pro	Phe	Phe	Val	Lys	Glu	Leu	Val	Val	Asn	Val	Cys	Asp
							305			310				315	320
Lys	Cys	Lys	Ile	Ser	Glu	Glu	Met	Ser	Asn	Phe	Leu	Ala	Trp	Leu	Gly
							325			330				335	
Tyr	Leu	Asn	Ser	Leu	Ile	Asn	Pro	Leu	Ile	Tyr	Thr	Ile	Phe	Asn	Glu
							340			345				350	
Asp	Phe	Lys	Lys	Ala	Phe	Gln	Lys	Leu	Val	Arg	Cys	Arg	Cys		
							355			360				365	

## (2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 422 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) IMMEDIATE SOURCE:  
(B) CLONE: 5-HT1A

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met	Asp	Val	Leu	Ser	Pro	Gly	Gln	Gly	Asn	Asn	Thr	Thr	Ser	Pro	Pro
1							5				10				15
Ala	Pro	Phe	Glu	Thr	Gly	Cly	Asn	Thr	Thr	Gly	Ile	Ser	Asp	Val	Thr
							20			25				30	
Val	Ser	Tyr	Gln	Val	Ile	Thr	Ser	Leu	Leu	Gly	Thr	Leu	Ile	Phe	
							35			40				45	
Cys	Ala	Val	Leu	Gly	Asn	Ala	Cys	Val	Val	Ala	Ala	Ile	Ala	Leu	Glu
							50			55				60	

-79-

Arg Ser Leu Gln Asn Val Ala Asn Tyr Leu Ile Gly Ser Leu Ala Val  
 65 70 75 80  
 Thr Asp Leu Met Val Ser Val Leu Val Leu Pro Met Ala Ala Leu Tyr  
 85 90 95  
 Gln Val Leu Asn Lys Trp Thr Leu Gly Gln Val Thr Cys Asp Leu Phe  
 100 105 110  
 Ile Ala Leu Asp Val Leu Cys Cys Thr Ser Ser Ile Leu His Leu Cys  
 115 120 125  
 Ala Ile Ala Leu Asp Arg Tyr Trp Ala Ile Thr Asp Pro Ile Asp Tyr  
 130 135 140  
 Val Asn Lys Arg Thr Pro Arg Arg Ala Ala Ala Leu Ile Ser Leu Thr  
 145 150 155 160  
 Trp Leu Ile Gly Phe Leu Ile Ser Ile Pro Pro Met Leu Gly Trp Arg  
 165 170 175  
 Thr Pro Glu Asp Arg Ser Asp Pro Asp Ala Cys Thr Ile Ser Lys Asp  
 180 185 190  
 His Gly Tyr Thr Ile Tyr Ser Thr Phe Gly Ala Phe Tyr Ile Pro Leu  
 195 200 205  
 Leu Leu Met Leu Val Leu Tyr Gly Arg Ile Phe Arg Ala Ala Arg Phe  
 210 215 220  
 Arg Ile Arg Lys Thr Val Lys Lys Val Glu Lys Thr Gly Ala Asp Thr  
 225 230 235 240  
 Arg His Gly Ala Ser Pro Ala Pro Gln Pro Lys Lys Ser Val Asn Gly  
 245 250 255  
 Glu Ser Gly Ser Arg Asn Trp Arg Leu Gly Val Glu Ser Lys Ala Gly  
 260 265 270  
 Gly Ala Leu Cys Ala Asn Gly Ala Val Arg Gln Gly Asp Asp Gly Ala  
 275 280 285  
 Ala Leu Glu Val Ile Glu Val His Arg Val Gly Asn Ser Lys Glu His  
 290 295 300  
 Leu Pro Leu Pro Ser Glu Ala Gly Pro Thr Pro Cys Ala Pro Ala Ser  
 305 310 315 320  
 Phe Glu Arg Lys Asn Glu Arg Asn Ala Glu Ala Lys Arg Lys Met Ala  
 325 330 335  
 Leu Ala Arg Glu Arg Lys Thr Val Lys Thr Leu Gly Ile Ile Met Gly  
 340 345 350  
 Thr Phe Ile Leu Cys Trp Leu Pro Phe Phe Ile Val Ala Leu Val Leu  
 355 360 365  
 Pro Phe Cys Glu Ser Ser Cys His Met Pro Thr Leu Leu Gly Ala Ile  
 370 375 380  
 Ile Asn Trp Leu Gly Tyr Ser Asn Ser Leu Leu Asn Pro Val Ile Tyr  
 385 390 395 400

-80-

Ala Tyr Phe Asn Lys Asp Phe Gln Asn Ala Phe Lys Lys Ile Ile Lys  
 405 410 415

Cys Leu Phe Cys Arg Gln  
 420

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 460 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vii) IMMEDIATE SOURCE:

- (B) CLONE: 5-HT1C

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Val Asn Leu Gly Asn Ala Val Arg Ser Leu Leu Met His Leu Ile  
 1 5 10 15

Gly Leu Leu Val Trp Gln Phe Asp Ile Ser Ile Ser Pro Val Ala Ala  
 20 25 30

Ile Val Thr Asp Thr Phe Asn Ser Ser Asp Gly Gly Arg Leu Phe Gln  
 35 40 45

Phe Pro Asp Gly Val Gln Asn Trp Pro Ala Leu Ser Ile Val Val Ile  
 50 55 60

Ile Ile Met Thr Ile Gly Gly Asn Ile Leu Val Ile Met Ala Val Ser  
 65 70 75 80

Met Glu Lys Lys Leu His Asn Ala Thr Asn Tyr Phe Leu Met Ser Leu  
 85 90 95

Ala Ile Ala Asp Met Leu Val Gly Leu Leu Val Met Pro Leu Ser Leu  
 100 105 110

Leu Ala Ile Leu Tyr Asp Tyr Val Trp Pro Leu Pro Arg Tyr Leu Cys  
 115 120 125

Pro Val Trp Ile Ser Leu Asp Val Leu Phe Ser Thr Ala Ser Ile Met  
 130 135 140

His Leu Cys Ala Ile Ser Leu Asp Arg Tyr Val Ala Ile Arg Asn Pro  
 145 150 155 160

Ile Glu His Ser Arg Phe Asn Ser Arg Thr Lys Ala Ile Met Lys Ile  
 165 170 175

-81-

Ala Ile Val Trp Ala Ile Ser Ile Gly Val Ser Val Pro Ile Pro Val  
 180 185 190  
 Ile Gly Leu Arg Asp Glu Ser Lys Val Phe Val Asn Asn Thr Thr Cys  
 195 200 205  
 Val Leu Asn Asp Pro Asn Phe Val Leu Ile Gly Ser Phe Val Ala Phe  
 210 215 220  
 Phe Ile Pro Leu Thr Ile Met Val Ile Thr Tyr Phe Leu Thr Ile Tyr  
 225 230 235 240  
 Val Leu Arg Arg Gln Thr Leu Met Leu Leu Arg Gly His Thr Glu Glu  
 245 250 255  
 Glu Leu Ala Asn Met Ser Leu Asn Phe Leu Asn Cys Cys Cys Lys Lys  
 260 265 270  
 Asn Gly Gly Glu Glu Asn Ala Pro Asn Pro Asn Pro Asp Gln Lys  
 275 280 285  
 Pro Arg Arg Lys Lys Glu Lys Arg Pro Arg Gly Thr Met Gln Ala  
 290 295 300  
 Ile Asn Asn Glu Lys Lys Ala Ser Lys Val Leu Gly Ile Val Phe Phe  
 305 310 315 320  
 Val Phe Leu Ile Met Trp Cys Pro Phe Phe Ile Thr Asn Ile Leu Ser  
 325 330 335  
 Val Leu Cys Gly Lys Ala Cys Asn Gln Lys Leu Met Glu Lys Leu Leu  
 340 345 350  
 Asn Val Phe Val Trp Ile Gly Tyr Val Cys Ser Gly Ile Asn Pro Leu  
 355 360 365  
 Val Tyr Thr Leu Phe Asn Lys Ile Tyr Arg Arg Ala Phe Ser Lys Tyr  
 370 375 380  
 Leu Arg Cys Asp Tyr Lys Pro Asp Lys Lys Pro Pro Val Arg Gln Ile  
 385 390 395 400  
 Pro Arg Val Ala Ala Thr Ala Leu Ser Gly Arg Glu Leu Asn Val Asn  
 405 410 415  
 Ile Tyr Arg His Thr Asn Glu Arg Val Ala Arg Lys Ala Asn Asp Pro  
 420 425 430  
 Glu Pro Gly Ile Glu Met Gln Val Glu Asn Leu Glu Leu Pro Val Asn  
 435 440 445  
 Pro Ser Asn Val Val Ser Glu Arg Ile Ser Ser Val  
 450 455 460

## (2) INFORMATION FOR SEQ ID NO:5:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 376 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: linear

-82-

- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: N-terminal
- (vii) IMMEDIATE SOURCE:  
(B) CLONE: 5-HT1DA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Ser Pro Leu Asn Gln Ser Ala Glu Gly Leu Pro Gln Glu Ala Ser  
1 5 10 15

Asn Arg Ser Leu Asn Ala Thr Glu Thr Ser Glu Ala Trp Asp Pro Arg  
20 25 30

Thr Leu Gln Ala Leu Lys Ile Ser Leu Pro Val Leu Leu Ser Val Ile  
35 40 45

Thr Leu Ala Thr Val Leu Ser Asn Ala Phe Val Leu Thr Thr Ile Leu  
50 55 60

Leu Thr Arg Lys Leu His Thr Pro Ala Asn Tyr Leu Ile Gly Ser Leu  
65 70 75 80

Ala Thr Thr Asp Leu Leu Val Ser Ile Leu Val Met Pro Ile Ser Met  
85 90 95

Ala Tyr Thr Ile Thr His Thr Trp Asn Phe Gly Gln Ile Leu Cys Asp  
100 105 110

Ile Trp Leu Ser Ser Asp Ile Thr Cys Cys Thr Ala Ser Ile Leu His  
115 120 125

Leu Cys Val Ile Ala Leu Asp Arg Tyr Trp Ala Ile Thr Asp Ala Leu  
130 135 140

Glu Tyr Ser Lys Arg Arg Thr Ala Gly His Ala Ala Thr Met Ile Ala  
145 150 155 160

Ile Val Trp Ala Ile Ser Ile Cys Ile Ser Ile Pro Pro Leu Phe Trp  
165 170 175

Arg Gln Glu Lys Ala Gln Glu Gln Met Ser Asp Cys Leu Val Asn Thr  
180 185 190

Ser Gln Ile Ser Tyr Thr Ile Tyr Ser Thr Cys Gly Ala Phe Tyr Ile  
195 200 205

Pro Ser Val Leu Leu Ile Ile Leu Tyr Gly Arg Ile Tyr Arg Ala Ala  
210 215 220

Arg Asn Arg Ile Leu Asn Pro Pro Ser Leu Ser Gly Lys Arg Phe Thr  
225 230 235 240

Thr Ala His Leu Ile Thr Gly Ser Ala Gly Ser Val Cys Ser Leu Asn  
245 250 255

-83-

Ser Ser Leu His Glu Gly His Ser His Ser Ala Gly Ser Pro Leu Phe  
 260 265 270  
 Phe Asn His Val Lys Ile Lys Leu Ala Asp Ser Ala Leu Glu Arg Lys  
 275 280 285  
 Arg Ile Ser Ala Ala Arg Glu Arg Lys Ala Thr Lys Ile Leu Gly Ile  
 290 295 300  
 Ile Leu Gly Ala Phe Ile Ile Cys Trp Leu Pro Phe Phe Val Val Ser  
 305 310 315 320  
 Leu Val Leu Pro Ile Cys Arg Asp Ser Cys Trp Ile His Pro Gly Leu  
 325 330 335  
 Phe Asp Phe Phe Thr Trp Leu Gly Tyr Leu Asn Ser Leu Ile Asn Pro  
 340 345 350  
 Ile Ile Tyr Thr Val Phe Asn Glu Glu Phe Arg Gln Ala Phe Gln Lys  
 355 360 365  
 Ile Val Pro Phe Arg Lys Ala Ser  
 370 375

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 390 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: unknown
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: N-terminal
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 5-HT1DB

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Glu Glu Pro Gly Ala Gln Cys Ala Pro Pro Ala Pro Ala Gly Ser  
 1 5 10 15  
 Glu Thr Trp Val Pro Gln Ala Asn Leu Ser Ser Ala Pro Ser Gln Asn  
 20 25 30  
 Cys Ser Ala Lys Asp Tyr Ile Tyr Gln Asp Ser Ile Ser Leu Pro Trp  
 35 40 45  
 Lys Val Leu Leu Val Met Leu Leu Ala Leu Ile Thr Leu Ala Thr Thr  
 50 55 60  
 Leu Ser Asn Ala Phe Val Ile Ala Thr Val Tyr Arg Thr Arg Lys Leu  
 65 70 75 80

-84-

His Thr Pro Ala Asn Tyr Leu Ile Ala Ser Leu Asp Val Thr Asp Leu  
 85 90 95  
 Leu Val Ser Ile Leu Val Ile Pro Ile Ser Thr Met Tyr Thr Val Thr  
 100 105 110  
 Asp Arg Trp Thr Leu Ser Gln Val Val Cys Asp Phe Trp Leu Ser Ser  
 115 120 125  
 Asp Ile Thr Cys Cys Thr Ala Ser Ile Leu His Leu Cys Val Ile Ala  
 130 135 140  
 Leu Asp Arg Tyr Trp Ala Ile Thr Asp Ala Val Glu Tyr Ser Ala Lys  
 145 150 155 160  
 Arg Thr Pro Lys Arg Ala Ala Val Met Ile Ala Leu Val Trp Val Phe  
 165 170 175  
 Ser Ile Ser Ile Ser Leu Pro Pro Phe Phe Trp Arg Gln Ala Lys Ala  
 180 185 190  
 Glu Glu Glu Val Ser Glu Cys Val Val Asn Thr Asp His Ile Leu Tyr  
 195 200 205  
 Thr Val Tyr Ser Thr Val Gly Ala Phe Tyr Phe Pro Thr Leu Leu Leu  
 210 215 220  
 Ile Ala Leu Tyr Gly Arg Ile Tyr Val Glu Ala Arg Ser Arg Ile Leu  
 225 230 235 240  
 Lys Gln Thr Pro Asn Arg Thr Gly Lys Arg Leu Thr Arg Ala Gln Leu  
 245 250 255  
 Ile Thr Asp Ser Pro Gly Ser Thr Ser Ser Val Thr Ser Ile Asn Ser  
 260 265 270  
 Arg Val Pro Asp Val Pro Ser Glu Ser Gly Ser Pro Val Tyr Val Asn  
 275 280 285  
 Gln Val Lys Val Arg Val Ser Asp Ala Leu Leu Glu Lys Lys Lys Leu  
 290 295 300  
 Met Ala Ala Arg Glu Arg Lys Ala Thr Lys Thr Leu Gly Ile Ile Leu  
 305 310 315 320  
 Gly Ala Phe Ile Val Cys Trp Leu Pro Phe Phe Ile Ile Ser Leu Val  
 325 330 335  
 Met Pro Ile Cys Lys Asp Ala Cys Trp Phe His Leu Ala Ile Phe Asp  
 340 345 350  
 Phe Phe Thr Trp Leu Gly Tyr Leu Asn Ser Leu Ile Asn Pro Ile Ile  
 355 360 365  
 Tyr Thr Met Ser Asn Glu Asp Phe Lys Gln Ala Phe His Lys Leu Ile  
 370 375 380  
 Arg Phe Lys Cys Thr Ser  
 385 390

(2) INFORMATION FOR SEQ ID NO:7:

-85-

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 366 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: unknown  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vii) IMMEDIATE SOURCE:  
(B) CLONE: 5-HT1F

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Asp Phe Leu Asn Ser Ser Asp Gln Asn Leu Thr Ser Glu Glu Leu  
1 5 10 15

Leu Asn Arg Met Pro Ser Lys Ile Leu Val Ser Leu Thr Leu Ser Gly  
20 25 30

Leu Ala Leu Met Thr Thr Ile Asn Ser Leu Val Ile Ala Ala Ile  
35 40 45

Ile Val Thr Arg Lys Leu His His Pro Ala Asn Tyr Leu Ile Cys Ser  
50 55 60

Leu Ala Val Thr Asp Phe Leu Val Ala Val Leu Val Met Pro Phe Ser  
65 70 75 80

Ile Val Tyr Ile Val Arg Glu Ser Trp Ile Met Gly Gln Val Val Cys  
85 90 95

Asp Ile Trp Leu Ser Val Asp Ile Thr Cys Cys Thr Cys Ser Ile Leu  
100 105 110

His Leu Ser Ala Ile Ala Leu Asp Arg Tyr Arg Ala Ile Thr Asp Ala  
115 120 125

Val Glu Tyr Ala Arg Lys Arg Thr Pro Lys His Ala Gly Ile Met Ile  
130 135 140

Thr Ile Val Trp Ile Ile Ser Val Phe Ile Ser Met Pro Pro Leu Phe  
145 150 155 160

Trp Arg His Gln Gly Thr Ser Arg Asp Asp Glu Cys Ile Ile Lys His  
165 170 175

Asp His Ile Val Ser Thr Ile Tyr Ser Thr Phe Gly Ala Phe Tyr Ile  
180 185 190

Pro Leu Ala Leu Ile Leu Ile Tyr Tyr Lys Ile Tyr Arg Ala Ala  
195 200 205

Lys Thr Leu Tyr His Lys Arg Gln Ala Ser Arg Ile Ala Lys Glu Glu  
210 215 220

-86-

Val Asn Gly Gln Val Leu Leu Glu Ser Gly Glu Lys Ser Thr Lys Ser  
 225 230 235 240  
 Val Ser Thr Ser Tyr Val Leu Glu Lys Ser Leu Ser Asp Pro Ser Thr  
 245 250 255  
 Asp Phe Asp Lys Ile His Ser Thr Val Arg Ser Leu Arg Ser Glu Phe  
 260 265 270  
 Lys His Glu Lys Ser Trp Arg Arg Gln Lys Ile Ser Gly Thr Arg Glu  
 275 280 285  
 Arg Lys Ala Ala Thr Thr Leu Gly Leu Ile Leu Gly Ala Phe Val Ile  
 290 295 300  
 Cys Trp Leu Pro Phe Phe Val Lys Glu Leu Val Val Asn Val Cys Asp  
 305 310 315 320  
 Lys Cys Lys Ile Ser Glu Glu Met Ser Asn Phe Leu Ala Trp Leu Gly  
 325 330 335  
 Tyr Leu Asn Ser Leu Ile Asn Pro Leu Ile Tyr Thr Ile Phe Asn Glu  
 340 345 350  
 Asp Phe Lys Lys Ala Phe Gln Lys Leu Val Arg Cys Arg Cys  
 355 360 365

## (2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 471 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: unknown  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

## (vii) IMMEDIATE SOURCE:

(B) CLONE: 5-HT2

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Asp Ile Leu Cys Glu Glu Asn Thr Ser Leu Ser Ser Thr Thr Asn  
 1 5 10 15  
 Ser Leu Met Gln Leu Asn Asp Asp Thr Arg Leu Tyr Ser Asn Asp Phe  
 20 25 30  
 Asn Ser Gly Glu Ala Asn Thr Ser Asp Ala Phe Asn Trp Thr Val Asp  
 35 40 45  
 Ser Glu Asn Arg Thr Asn Leu Ser Cys Glu Gly Cys Leu Ser Pro Ser  
 50 55 60

-87-

Cys Leu Ser Leu Leu His Leu Gln Glu Lys Asn Trp Ser Ala Leu Leu  
 65 70 75 80

Thr Ala Val Val Ile Ile Leu Thr Ile Ala Gly Asn Ile Leu Val Ile  
 85 90 95

Met Ala Val Ser Leu Glu Lys Lys Leu Gln Asn Ala Thr Asn Tyr Phe  
 100 105 110

Leu Met Ser Leu Ala Ile Ala Asp Met Leu Leu Gly Phe Leu Val Met  
 115 120 125

Pro Val Ser Met Leu Thr Ile Leu Tyr Gly Tyr Arg Trp Pro Leu Pro  
 130 135 140

Ser Lys Leu Cys Ala Val Trp Ile Tyr Leu Asp Val Leu Phe Ser Thr  
 145 150 155 160

Ala Ser Ile Met His Leu Cys Ala Ile Ser Leu Asp Arg Tyr Val Ala  
 165 170 175

Ile Gln Asn Pro Ile His His Ser Arg Phe Asn Ser Arg Thr Lys Ala  
 180 185 190

Phe Leu Lys Ile Ile Ala Val Trp Thr Ile Ser Val Gly Ile Ser Met  
 195 200 205

Pro Ile Pro Val Phe Gly Leu Gln Asp Asp Ser Lys Val Phe Lys Glu  
 210 215 220

Gly Ser Cys Leu Leu Ala Asp Asp Asn Phe Val Leu Ile Gly Ser Phe  
 225 230 235 240

Val Ser Phe Phe Ile Pro Leu Thr Ile Met Val Ile Thr Tyr Phe Leu  
 245 250 255

Thr Ile Lys Ser Leu Gln Lys Glu Ala Thr Leu Cys Val Ser Asp Leu  
 260 265 270

Gly Thr Arg Ala Lys Leu Ala Ser Phe Ser Phe Leu Pro Gln Ser Ser  
 275 280 285

Leu Ser Ser Glu Lys Leu Phe Gln Arg Ser Ile His Arg Glu Pro Gly  
 290 295 300

Ser Tyr Thr Gly Arg Arg Thr Met Gln Ser Ile Ser Asn Glu Gln Lys  
 305 310 315 320

Ala Cys Lys Val Leu Gly Ile Val Phe Phe Leu Phe Val Val Met Trp  
 325 330 335

Cys Pro Phe Phe Ile Thr Asn Ile Met Ala Val Ile Cys Lys Glu Ser  
 340 345 350

Cys Asn Glu Asp Val Ile Gly Ala Leu Leu Asn Val Phe Val Trp Ile  
 355 360 365

Gly Tyr Leu Ser Ser Ala Val Asn Pro Leu Val Tyr Thr Leu Phe Asn  
 370 375 380

Lys Thr Tyr Arg Ser Ala Phe Ser Arg Tyr Ile Gln Cys Gln Tyr Lys  
 385 390 395 400

-86-

Glu Asn Lys Lys Pro Leu Gln Leu Ile Leu Val Asn Thr Ile Pro Ala  
405 410 415

Leu Ala Tyr Lys Ser Ser Gln Leu Gln Met Gly Gln Lys Lys Asn Ser  
420 425 430

Lys Gln Asp Ala Lys Thr Thr Asp Asn Asp Cys Ser Met Val Ala Leu  
435 440 445

Gly Lys Gln His Ser Glu Glu Ala Ser Lys Asp Asn Ser Asp Gly Val  
450 455 460

Asn Glu Lys Val Ser Cys Val  
465 470

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: ANTISENSE OLIGO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TCTCACCACT CTCCAAAAGG ACTTGGCCAT TCACCTCCTC CTTTG

45

-89-

What is claimed is:

1. An isolated nucleic acid molecule encoding a human 5-HT<sub>1F</sub> receptor.
- 5 2. An isolated nucleic acid molecule of claim 1, wherein the nucleic acid molecule is a DNA molecule.
- 10 3. An isolated DNA molecule of claim 2, wherein the DNA molecule is a cDNA molecule encoding a human 5-HT<sub>1F</sub> receptor.
4. An isolated human 5-HT<sub>1F</sub> receptor protein.
- 15 5. A vector comprising the DNA molecule of claim 2.
6. A plasmid comprising the vector of claim 5.
- 20 7. A vector of claim 5 adapted for expression in a bacterial cell which comprises the regulatory elements necessary for expression of the DNA in the bacterial cell so located relative to the DNA encoding the 5-HT<sub>1F</sub> receptor as to permit expression thereof.
- 25 8. A vector of claim 5 adapted for expression in a yeast cell which comprises the regulatory elements necessary for expression of the DNA in the yeast cell so located relative to the DNA encoding the 5-HT<sub>1F</sub> receptor as to permit expression thereof.
- 30 9. A vector of claim 5 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding the 5-HT<sub>1F</sub> receptor as to permit expression thereof.
- 35

-90-

10. A plasmid of claim 6 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding the 5-HT<sub>1F</sub> receptor as to permit expression thereof.
11. A plasmid comprising the cDNA molecule of claim 3 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the cDNA molecule as to permit expression thereof, designated pMOS-h116a (ATCC Accession No. 75175).
12. A mammalian cell comprising the plasmid of claim 6.
13. An Ltk<sup>-</sup> cell comprising the plasmid of claim 6.
14. An NIH3T3 cell comprising the plasmid of claim 6.
15. An Ltk<sup>-</sup> cell comprising the plasmid of claim 11, designated L-5-HT<sub>1F</sub> (ATCC Accession No. CRL 10957).
16. An NIH3T3 cell comprising the plasmid of claim 11 designated N-5-HT<sub>1F</sub> (ATCC Accession No. CRL 10956).
17. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human 5-HT<sub>1F</sub> receptor.
18. The nucleic acid probe of claim 17 wherein the nucleic acid is DNA .
19. An antisense oligonucleotide having a sequence

capable of binding specifically to an mRNA molecule encoding a human 5-HT<sub>1F</sub> receptor so as to prevent translation of the mRNA molecule.

- 5 20. An antisense oligonucleotide having a sequence capable of binding specifically to the cDNA molecule of claim 3.
- 10 21. An antisense oligonucleotide of claim 19 comprising chemical analogues of nucleotides.
22. An antibody directed to a human 5-HT<sub>1F</sub> receptor.
- 15 23. A monoclonal antibody directed to an epitope of a human 5-HT<sub>1F</sub> receptor present on the surface of a cell and having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human 5-HT<sub>1F</sub> receptor.
- 20 24. A pharmaceutical composition comprising an amount of the oligonucleotide of claim 19 effective to reduce expression of a human 5-HT<sub>1F</sub> receptor by passing through a cell membrane and binding specifically with mRNA encoding a human 5-HT<sub>1F</sub> receptor in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane.
- 25 30 25. A pharmaceutical composition of claim 24, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.
- 35 26. A pharmaceutical composition of claim 25, wherein the substance which inactivates mRNA is a ribozyme.
27. A pharmaceutical composition of claim 24, wherein the pharmaceutically acceptable hydrophobic carrier

-92-

capable of passing through a cell membrane comprises a structure which binds to a receptor specific for a selected cell type and is thereby taken up by cells of the selected cell type.

5

28. A pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a human 5-HT<sub>1F</sub> receptor and a pharmaceutically acceptable carrier.

10

29. A pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of 5-HT<sub>1F</sub> receptor and a pharmaceutically acceptable carrier.

15

30. A pharmaceutical composition which comprises an amount of the antibody of claim 22 effective to block binding of naturally occurring ligands to the 5-HT<sub>1F</sub> receptor and a pharmaceutically acceptable carrier.

20

31. A transgenic nonhuman mammal expressing DNA encoding a human 5-HT<sub>1F</sub> receptor.

25

32. A transgenic nonhuman mammal expressing DNA encoding a human 5-HT<sub>1F</sub> receptor so mutated as to be incapable of normal receptor activity, and not expressing native 5-HT<sub>1F</sub> receptor.

30

33. A transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a human 5-HT<sub>1F</sub> receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a 5-HT<sub>1F</sub> receptor and which hybridizes to mRNA encoding a 5-HT<sub>1F</sub> receptor thereby reducing its translation.

35

34. The transgenic nonhuman mammal of any of claims 31, 32 or 33, wherein the DNA encoding a human 5-HT<sub>1F</sub> receptor additionally comprises an inducible promoter.

5

35. The transgenic nonhuman mammal of any of claims 31, 32 or 33, wherein the DNA encoding a human 5-HT<sub>1F</sub> receptor additionally comprises tissue specific regulatory elements.

10

36. A transgenic nonhuman mammal of any of claims 31, 32 or 33, wherein the transgenic nonhuman mammal is a mouse.

15

37. A method for determining whether a ligand not known to be capable of binding to a human 5-HT<sub>1F</sub> receptor can bind to a human 5-HT<sub>1F</sub> receptor which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a human 5-HT<sub>1F</sub> receptor with the ligand under conditions permitting binding of ligands known to bind to a 5-HT<sub>1F</sub> receptor, detecting the presence of any of the ligand bound to a human 5-HT<sub>1F</sub> receptor, and thereby determining whether the ligand binds to a human 5-HT<sub>1F</sub> receptor.

20

25

38. A method for determining whether a ligand not known to be capable of binding to the human 5-HT<sub>1F</sub> receptor can functionally activate receptor activity or prevent the action of a ligand which does so comprising contacting a mammalian cell of claim 12 with the ligand under conditions permitting the activation or blockade of a functional response, and detecting by means of a bioassay from the mammalian cell such as a second messenger response, and thereby determining whether the ligand activates or prevents the activation of the human 5-HT<sub>1F</sub> receptor functional output.

30

35

-94-

39. The method of claim 37 or 38 wherein the mammalian cell is nonneuronal in origin.
40. A method of claim 39, wherein the mammalian cell nonneuronal in origin is an Ltk<sup>-</sup> cell.  
5
41. A method of claim 39, wherein the mammalian cell nonneuronal in origin is an NIH3T3 cell.
- 10 42. A ligand detected by the method of claim 37 or 38.
43. A method of screening drugs to identify drugs which specifically interact with, and bind to, the human 5-HT<sub>1F</sub> receptor on the surface of a cell which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a human 5-HT<sub>1F</sub> receptor with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, a human 5-HT<sub>1F</sub> receptor.  
15
- 20 44. A method of screening drugs to identify drugs which interact with, and activate or block the activation of, the human 5-HT<sub>1F</sub> receptor on the surface of a cell which comprises contacting the mammalian cell of claim 12 with a plurality of drugs, determining those drugs which activate or block the activation of the receptor in the mammalian cell using a bioassay such as a second messenger assays, and thereby identifying drugs which specifically interact with, and activate or block the activation of, a human 5-HT<sub>1F</sub> receptor.  
25
- 30 45. The method of claim 43 or 44 wherein the mammalian cell is nonneuronal in origin.  
35
46. The method of claim 45 wherein the mammalian cell

-95-

nonneuronal in origin is an Ltk<sup>-</sup> cell.

47. The method of claim 45 wherein the mammalian cell nonneuronal in origin is an NIH3T3 cell.

5

48. A pharmaceutical composition comprising a drug identified by the method of claim 43 or 44 and a pharmaceutically acceptable carrier.

10 49. A method of detecting expression of the 5-HT<sub>1F</sub> receptor on the surface of a cell by detecting the presence of mRNA coding for a 5-HT<sub>1F</sub> receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the nucleic acid probe of claim 17 under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the 5-HT<sub>1F</sub> receptor by the cell.

15

20 50. A method of treating abnormalities in a subject, wherein the abnormality is alleviated by the reduced expression of a 5-HT<sub>1F</sub> receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 24 effective to reduce expression of the 5-HT<sub>1F</sub> receptor by the subject.

25

30 51. A method of treating an abnormal condition related to an excess of 5-HT<sub>1F</sub> receptor activity which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 24 effective to reduce expression of the 5-HT<sub>1F</sub> receptor in the subject.

35 52. The method of claim 51 wherein the abnormal condition is dementia.

-96-

53. The method of claim 51 wherein the abnormal condition Parkinson's disease.
- 5 54. The method of claim 51 wherein the abnormal condition is a feeding disorder.
- 10 55. The method of claim 51 wherein the abnormal condition is pathological anxiety.
- 15 56. The method of claim 51 wherein the abnormal condition is schizophrenia.
- 20 57. The method of claim 51 wherein the abnormal condition is a migraine headache.
- 25 58. A method of treating abnormalities which are alleviated by reduction of expression of a human 5-HT<sub>1F</sub> receptor which comprises administering to a subject an amount of the pharmaceutical composition of claim 30 effective to block binding of naturally occurring ligands to the 5-HT<sub>1F</sub> receptor and thereby alleviate abnormalities resulting from overexpression of a human 5-HT<sub>1F</sub> receptor.
- 30 59. A method of treating an abnormal condition related to an excess of 5-HT<sub>1F</sub> receptor activity which comprises administering to a subject an amount of the pharmaceutical composition of claim 30 effective to block binding of naturally occurring ligands to the 5-HT<sub>1F</sub> receptor and thereby alleviate the abnormal condition.
- 35 60. The method of claim 59 wherein the abnormal condition is dementia.
61. The method of claim 59 wherein the abnormal condition is Parkinson's disease.

-97-

62. The method of claim 59 wherein the abnormal condition is a feeding disorder.
- 5 63. The method of claim 59 wherein the abnormal condition is a pathological anxiety.
64. The method of claim 59 wherein the abnormal condition is schizophrenia.
- 10 65. The method of claim 59 wherein the abnormal condition is a migraine headache.
- 15 66. A method of detecting the presence of a human 5-HT<sub>1F</sub> receptor on the surface of a cell which comprises contacting the cell with the antibody of claim 22 under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a human 5-HT<sub>1F</sub> receptor on the surface of the cell.
- 20 67. A method of determining the physiological effects of expressing varying levels of human 5-HT<sub>1F</sub> receptors which comprises producing a transgenic nonhuman mammal whose levels of human 5-HT<sub>1F</sub> receptor expression are varied by use of an inducible promoter which regulates human 5-HT<sub>1F</sub> receptor expression.
- 25 68. A method of determining the physiological effects of expressing varying levels of human 5-HT<sub>1F</sub> receptors which comprises producing a panel of transgenic nonhuman mammals each expressing a different amount of human 5-HT<sub>1F</sub> receptor.
- 30 69. A method for identifying a substance capable of alleviating the abnormalities resulting from

5 overexpression of a human 5-HT<sub>1F</sub> receptor comprising administering a substance to the transgenic nonhuman mammal of claim 31 and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overexpression of a human 5-HT<sub>1F</sub> receptor.

10 70. A method for treating the abnormalities resulting from overexpression of a human 5-HT<sub>1F</sub> receptor which comprises administering to a subject an amount of the pharmaceutical composition of claim 28 effective to alleviate the abnormalities resulting from overexpression of a human 5-HT<sub>1F</sub> receptor.

15 71. A method for identifying a substance capable of alleviating the abnormalities resulting from underexpression of a human 5-HT<sub>1F</sub> receptor comprising administering the substance to the transgenic nonhuman mammal of either of claims 32 or 20 33 and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of underexpression of a human 5-HT<sub>1F</sub> receptor.

25 72. A method for treating the abnormalities resulting from underexpression of a human 5-HT<sub>1F</sub> receptor which comprises administering to a subject an amount of the pharmaceutical composition of claim 29 effective to alleviate the abnormalities resulting from underexpression of a human 5-HT<sub>1F</sub> receptor.

30 73. A method for diagnosing a predisposition to a disorder associated with the expression of a specific human 5-HT<sub>1F</sub> receptor allele which 35 comprises:

-99-

- a. obtaining DNA of subjects suffering from the disorder;
- 5 b. performing a restriction digest of the DNA with a panel of restriction enzymes;
- c. electrophoretically separating the resulting DNA fragments on a sizing gel;
- 10 d. contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human 5-HT<sub>1F</sub> receptor and labelled with a detectable marker;
- 15 e. detecting labelled bands which have hybridized to the DNA encoding a human 5-HT<sub>1F</sub> receptor labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder;
- 20 f. preparing DNA obtained for diagnosis by steps a-e; and
- 25 g. comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.
- 30 74. The method of claim 73 wherein a disorder associated with the expression of a specific human 5-HT<sub>1F</sub> receptor allele is diagnosed.
- 35 75. A method of preparing the isolated 5-HT<sub>1F</sub> receptor of claim 4 which comprises:

-100-

- a. inducing cells to express 5-HT<sub>1F</sub> receptor;
- b. recovering the receptor from the resulting cells; and
- 5 c. purifying the receptor so recovered.

76. A method of preparing the isolated 5-HT<sub>1F</sub> receptor of claim 4 which comprises:

- 10 a. inserting nucleic acid encoding 5-HT<sub>1F</sub> receptor in a suitable vector;
- b. inserting the resulting vector in a suitable host cell;
- 15 c. recovering the receptor produced by the resulting cell; and
- d. purifying the receptor so recovered.

1/14

FIGURE 1A

TGCATCCCTCAGGTCTAGGATCCCCGGTACCGAGCTCGAATTCCTTGT  
 -590 -570  
 -550 -530 -510  
 ATTTGTCATGCTTCAAGCCTAGGAAAGCCTAAGCATAAGCTGGCTCTATCAGCTTGAATATAACTCAACTA  
 -490 -470 -450  
 ACATTCCAGCCTTGAATAAGGGCACTGGCTCTATCAGCTTGAATATAACTCAACTA  
 -430 -410 -390  
 GTCAGTCACTGAAACAGTTACGGAGGCCTGCCTATTGAGATCGGGCCTGC  
 -370 -350 -330  
 CACACTTTAAACTTTCTGACATGGACAGAGAGAAACATTCTATAATGGCAGAC  
 -310 -290 -270  
 ATTCACTGAGTAACAAAGCTAGAGTATCATTAAGTATTAACTTAAACCTATTTT

SUBSTITUTE SHEET

2/14

FIGURE 1B

5' ←

-250                    -230                    -210

AGAAATGTTTGGAAAGTTACTGGCTTTTACGTCTCATTAATTTCTTAATTAATAA

-190                    -170                    -150

AGGAAAACTAAACCTTCAATCTGAACCTCATTTTTAATCTATAAATCTGGCTA

-130                    -110                    -90

AACATTAACATACACTTTAAATTATTCTGAAAGGAAGAACGTTCTGAAGGCCRT

-70                    -50                    -30

CTCTGAACTGTTTCTCTCCCTTGTACAGGTATCTGATCAAAACTGACCTCAGAGGAA

-10                    10                    30

TTTAAACAAAGAAATGGATTCTTAATTCAATCTGATCAAAACTGACCTCAGAGGAA

M D F L N S S D Q N L T S E E

## FIGURE 1C

50

70

90

CTGTTAACAGAACATGCCAAATTCTGGTGTCCCTCACTCTGTCTGGCTGGCACTG

L L N R M P S K I L V S L T L S G L A L

110

130

150

ATGACACAACTATCAAATCCCTTGTGATGCCATTATTGTGACCCCCGAAGCTGCAC

M T T T I N S L V I A A I I V T R K L H

170

190

210

CATCCAGCCATTATTAAATTCTGGCACTCACAGATTCTTGTGGCTGTCCCTG

H P A N Y L I C S L A V T D F L V A V L

230

250

270

GTGATGCCCTCAGCATTGTGTATATTGTGAGAGAGCTGGATTATGGCCAAAGTGGTC

V M P F S I V Y I V R E S W I M G Q V V

290

310

330

TGTGACATTTGGCTGACTGTTGACATTACCTGCTGCACCGTGGCTCCATCTGCATCTCA

C D I W L S V D I T C C T C S I L H L S

## FIGURE 1D

350	370	390	
A I A L D R Y R A I T D A V E Y A R K R			GCTATAGCTTGGATCGGTATCGAGCAATCACAGATGCTGTTGACTATGCCAGGAAAGG
			ACTCCAAGCATGCTGGCATTTATGATTACAATAAGTTGGATTATATCTGTTTTATCTCT
410	430	450	T P K H A G I M I T I V W I I S V F I S
			ATGCCTCTTATTCTGGAGGCACCAAGGAACTAGCAGAGATGATGAAATGCATCATCAAG
470	490	510	M P P L F W R H Q G T S R D D E C I I K
			CACGACCACTTGTCCACCATTTACTCAACATTGGAGCTTCTACATCCCACTGGCA
530	550	570	H D H I V S T I Y S T F G A F Y I P L A

FIGURE 1E

## SUBSTITUTE SHEET

6/14

## FIGURE 1F

890 910 930  
GGATTAAATCTGGGTGCATTGTAAATATGTTGGCTTCCCTTTGTAAAAGAATTAGTT  
G L I L G A F V I C W L P F F V K E L V  
950 970 990  
GTTAAATGCTGACAAATGTAATTCTGAAGAAATGTCCAAATTGTCATTGGCATGGCTT  
V N V C D K C K I S E E M S N F L A W L  
1010 1030 1050  
GGGTATCTCAATTCCCTTATAAATCCACTGATTACACAAATCTTTAATGAAGACTTCAG  
G Y L N S L I N P L I Y T I F N E D F K  
1070 1090 1110  
AAAGCATTCCAAAAGCTTGTGGATGTCGATGTTAGTTAAATTGTT  
K A F Q K L V R C R C

7/14

## FIGURE 2A

8/14

FIGURE 2B

151	<b>5HT<sub>1c</sub></b> <b>5HT<sub>2</sub></b> <b>5HT<sub>10a</sub></b> <b>5HT<sub>10b</sub></b> <b>5HT<sub>11</sub></b> <b>5HT<sub>14</sub></b> <b>TM Region</b>	<b>PWISILDVLF</b> <b>AVWIYLDVLF</b> <b>DIWLSSDITC</b> <b>DFWLSSDITC</b> <b>DIWLSVDITC</b> <b>DLFIALDVL</b> <b>.</b>	<b>STASIMHLCA</b> <b>STASIMHLCA</b> <b>CTASILHLCV</b> <b>CTASILHLCV</b> <b>CTCSILHLSA</b> <b>CTSSILHLCA</b> <b>.</b>	<b>ISLDRYVAIR</b> <b>ISLDRYVAIR</b> <b>IALDRYWAIT</b> <b>IALDRYWAIT</b> <b>IALDRYWAIT</b> <b>IALDRYWAIT</b> <b>.</b>	<b>NPIEHSRFNS</b> <b>NPIHHSRFNS</b> <b>DALEYSKRT</b> <b>DAVEYSAKRT</b> <b>DAVEYARKRT</b> <b>DPIDYVNKRT</b> <b>.</b>	<b>RTKAIMKIAI</b> <b>RTKAFLKIIA</b> <b>AGHAATMIAI</b> <b>PKRAAVMIAL</b> <b>PKHAGIMI</b> <b>PRRAAAILSL</b> <b>.</b>
201	<b>5HT<sub>1c</sub></b> <b>5HT<sub>2</sub></b> <b>5HT<sub>10a</sub></b> <b>5HT<sub>10b</sub></b> <b>5HT<sub>11</sub></b> <b>5HT<sub>14</sub></b> <b>TM Region</b>	<b>VWAISIGVSV</b> <b>VWTISVGISM</b> <b>VWAISICISI</b> <b>VWVFISISI</b> <b>VWIISVFISM</b> <b>TWLIGFLISI</b> <b>*</b>	<b>PIPVIGLRDE</b> <b>PIPVFGLQDD</b> <b>PILF.WRQA</b> <b>P.PFF.WRQA</b> <b>P.PLF.WRIQ</b> <b>P.PMIGWRTP</b> <b>IV..*</b>	<b>SKVFVNNTTC</b> <b>SKVF</b> <b>KEGSC</b> <b>VNTSQISYTI</b> <b>VNTDHILYTV</b> <b>YSTFGAFYIP</b> <b>GYTI</b>	<b>VLN</b> <b>NDP.</b> <b>NFVL</b> <b>IGSFV</b> <b>AFFFIP</b> <b>YVSTI</b> <b>YSTV</b> <b>GYTI</b>	<b>*</b>
251	<b>5HT<sub>1c</sub></b> <b>5HT<sub>2</sub></b> <b>5HT<sub>10a</sub></b> <b>5HT<sub>10b</sub></b> <b>5HT<sub>11</sub></b> <b>5HT<sub>14</sub></b> <b>TM Region</b>	<b>VWAISIGVSV</b> <b>VWTISVGISM</b> <b>VWAISICISI</b> <b>VWVFISISI</b> <b>VWIISVFISM</b> <b>TWLIGFLISI</b> <b>*</b>	<b>PIPVIGLRDE</b> <b>PIPVFGLQDD</b> <b>PILF.WRQA</b> <b>P.PFF.WRQA</b> <b>P.PLF.WRIQ</b> <b>P.PMIGWRTP</b> <b>IV..*</b>	<b>SKVFVNNTTC</b> <b>SKVF</b> <b>KEGSC</b> <b>VNTSQISYTI</b> <b>VNTDHILYTV</b> <b>YSTFGAFYIP</b> <b>GYTI</b>	<b>*</b>	<b>*</b>
300						

9/14

## FIGURE 2C

5HT <sub>1c</sub>	LTMIVITYFL	TI.....YVL	RRQTLMLRG	HTEEEELANN	SINFLNCCCK
5HT <sub>2</sub>	LTMIVITYFL	TI.....KSL	QKEATLCVSD	LGTRAKLA..	SFSFLPQSSL
5HT <sub>10<math>\alpha</math></sub>	SVLLILLYGR	TYRAARNRIL	NPPSLYGR	FTTAHLITGS	G..SSLCSDL
5HT <sub>10<math>\beta</math></sub>	TLLILLYGR	TYYEARSRL	KOTPNTGKR	LTRAQLITDS	PGSTSSVTSI
5HT <sub>1f</sub>	LALILLYK	TYRAAKTLYH	KRQASRIAKE	EVNGQVLLES	GE..KSTKSV
5HT <sub>1<math>\nu</math></sub>	LILMLVLYGR	IFRAARFRIR	KTVKKVEKTG	ADTRHGASPA	PQPKKSVNGE
TM Region	*****	.....	.....	.....	.....
			350		
5HT <sub>1c</sub>	KNGGEENAP	NPNPDDQ..KP	RRKKKEKRPR	GTMQ..	.....
5HT <sub>2</sub>	SSEKLFQRSI	HREPGS..YT	GRR..	TMQ..	.....
5HT <sub>10<math>\alpha</math></sub>	NSS..LHEGH	SHSAGSPLFF	N..HVKIKLAD	SALE..	.....
5HT <sub>10<math>\beta</math></sub>	NSR..VPDVP	SES..GSPVYY	N..QVKVRVSD	ALLE..	.....
5HT <sub>1f</sub>	STS..YVLEK	SLSDPSTDFF	KIHSTVRSLR	SEFKHEKSWR	.....
5HT <sub>1<math>\nu</math></sub>	SGSRNWRILGV	ESKAGGALCA	NGAVRQGDDG	AALEVIEVIR	VGNSKEHLPL
TM Region	.....	.....	.....	.....	.....
			351		
5HT <sub>1c</sub>	.....	.....	.....	AI	NNEKKASKVL
5HT <sub>2</sub>	.....	.....	.....	SI	SNEQKACKVL
5HT <sub>10<math>\alpha</math></sub>	.....	.....	.....	RKRISA	ARERKATKIL
5HT <sub>10<math>\beta</math></sub>	.....	.....	.....	KKKLMA	ARERKATKTL
5HT <sub>1f</sub>	.....	.....	.....	RQKISG	TRERKAATTI
5HT <sub>1<math>\nu</math></sub>	PSEAGPTPCA	PASFERNER	NAEAKRKMAL	ARERKTVKTL	GIIMGTFILC
TM Region	.....	.....	.....	.....	*****
			352		
5HT <sub>1c</sub>	.....	.....	.....	AI	NNEKKASKVL
5HT <sub>2</sub>	.....	.....	.....	SI	SNEQKACKVL
5HT <sub>10<math>\alpha</math></sub>	.....	.....	.....	RKRISA	ARERKATKIL
5HT <sub>10<math>\beta</math></sub>	.....	.....	.....	KKKLMA	ARERKATKTL
5HT <sub>1f</sub>	.....	.....	.....	RQKISG	TRERKAATTI
5HT <sub>1<math>\nu</math></sub>	PSEAGPTPCA	PASFERNER	NAEAKRKMAL	ARERKTVKTL	GIIMGTFILC
TM Region	.....	.....	.....	.....	*****

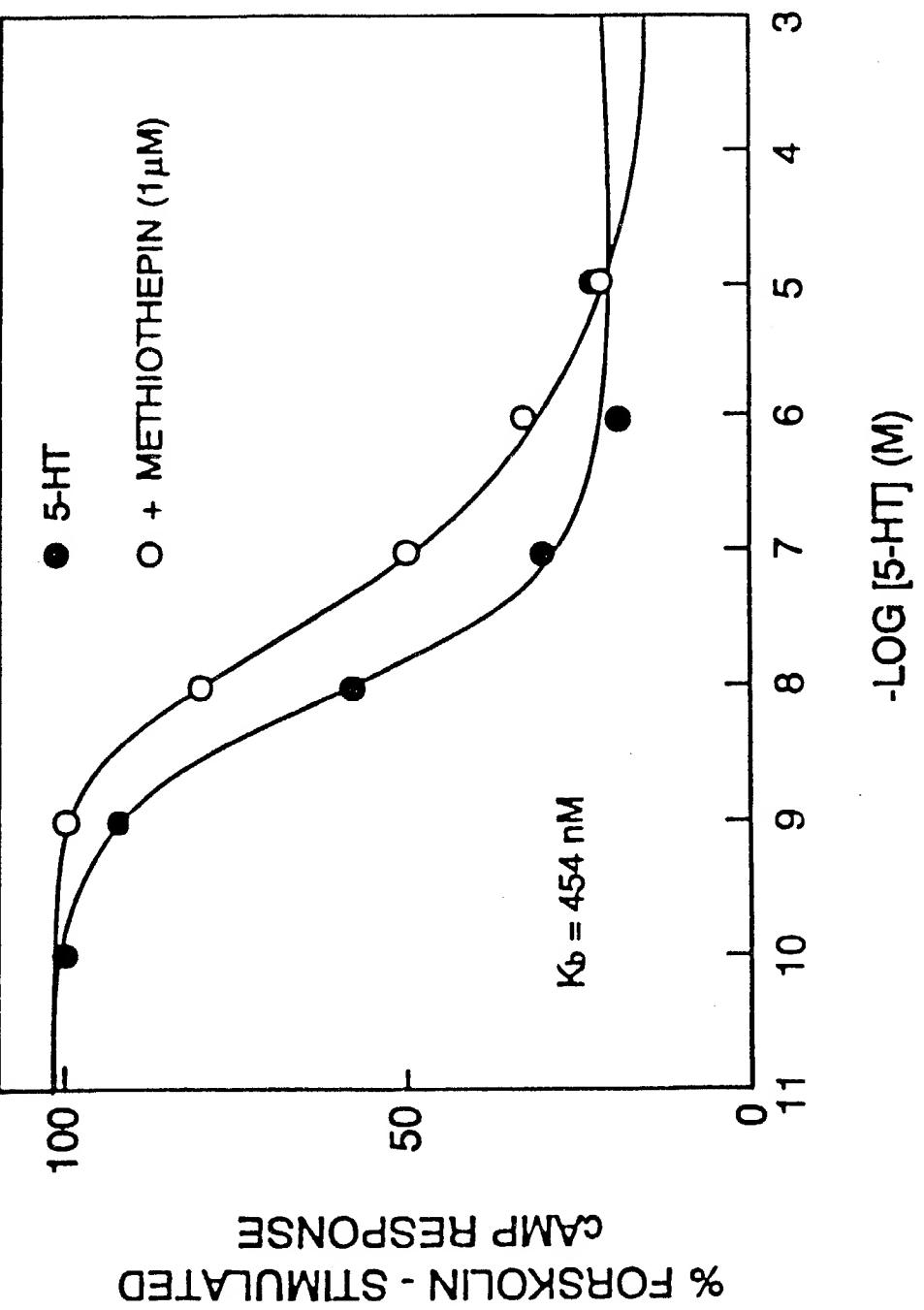
10/14

## FIGURE 2D

TM Region	401					450					500					551					600					
	5HT <sub>1c</sub>	5HT <sub>2</sub>	5HT <sup>10*</sup>	5HT <sup>10*</sup>	5HT <sub>1f</sub>	5HT <sub>1a</sub>	5HT <sub>1c</sub>	5HT <sub>2</sub>	5HT <sup>10*</sup>	5HT <sup>10*</sup>	5HT <sub>1f</sub>	5HT <sub>1a</sub>	5HT <sub>1c</sub>	5HT <sub>2</sub>	5HT <sup>10*</sup>	5HT <sup>10*</sup>	5HT <sub>1f</sub>	5HT <sub>1a</sub>	5HT <sub>1c</sub>	5HT <sub>2</sub>	5HT <sup>10*</sup>	5HT <sup>10*</sup>	5HT <sub>1f</sub>	5HT <sub>1a</sub>		
5HT <sub>1c</sub>	WCPFFITNIL	SVLCGKACNQ	KIMEKLLNVF	VWIGYVCSGI	NPLVYTLFNK																					
5HT <sub>2</sub>	WCPFFITNIM	AVICKESCNE	DVIGALLNVF	VWIGYLSAV	NPLVYTLFNK																					
5HT <sup>10*</sup>	WLPFFVVSIL	LPICRDSW.	IPGGLDFF	TWLGYLNSLI	NPIIYTDFNE																					
5HT <sup>10*</sup>	WLPFFFLISLV	MPICKDACK.	FHLAIFDFF	TWLGYLNSLI	NPIIYTMSNE																					
5HT <sub>1f</sub>	WLPFFVKEVL	VNVC.DKCK.	ISEEMSNFL	AWLGYLNSLI	NPLIYTIFNE																					
5HT <sub>1a</sub>	WLPFFFIVALV	LPFCESSCH.	MPTLIGAI	NWLGYNSNLL	NPVIYAYFNK																					
TM Region	V.I..*	**	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	
5HT <sub>1c</sub>	IYRRAFSKYL	RCDYKPKKP	PVRQIPRVA	ATALSGRELN	VNIYRHTNER																					
5HT <sub>2</sub>	TYRSAFSRYI	QCQYKENKKP	LQLLIVNTIP	ALAYKSSQLQ	MGQKQNSKQD																					
5HT <sup>10*</sup>	EFRQAFQKIV	PFRKAS.	..	..	..																					
5HT <sup>10*</sup>	DFKQAFHKLI	RFICCTS.	..	..	..																					
5HT <sub>1f</sub>	DFKKAFQKLV	RCRC.	..	..	..																					
5HT <sub>1a</sub>	DFQNAFKKII	KCLFCRQ.	..	..	..																					
TM Region	..	..	..	..	..																					
5HT <sub>1c</sub>	VARKANDPEP	GIEMQVENLE	LPVNPSNVVS	ERISSV.																						
5HT <sub>2</sub>	AKTTDNDCSM	VALGKQHSEE	ASKDNDGVN	EKVSCV.																						
5HT <sup>10*</sup>	..	..	..	..	..																					
5HT <sup>10*</sup>	..	..	..	..	..																					
5HT <sub>1f</sub>	..	..	..	..	..																					
5HT <sub>1a</sub>	..	..	..	..	..																					
TM Region	..	..	..	..	..																					

11/14

FIGURE 3



12/14

## FIGURE 4

Brain

Testes

Endometrium

Mesentery

Myometrium

Pancreas

Kidney

Liver

Spleen

Heart

 $H_2O$ 

Control



13/14

FIGURE 5A

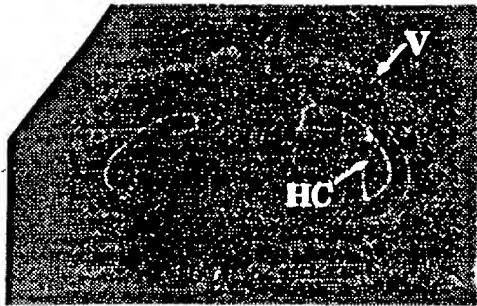


FIGURE 5B

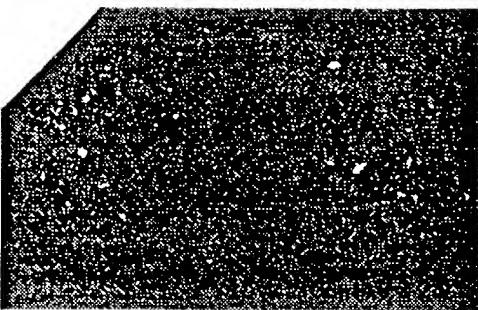
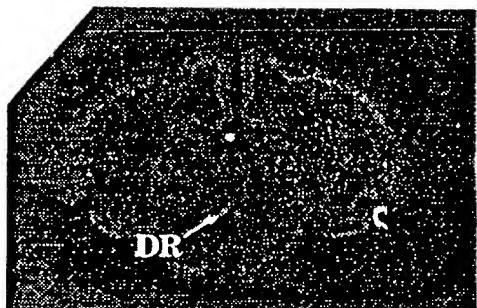


FIGURE 5C



14/14

FIGURE 5D



FIGURE 5E



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US93/00149

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.8; 435/6, 7.1, 69.1, 172.3, 240.2, 252.3, 252.33, 320.1; 514/12, 44; 530/350, 387.1, 388.1; 536/23.1, 23.4, 23.5; 800/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

World Patent Index, Biosis, Medline, Biotechnology Abstracts, APS, search terms: serotonin, 5-hydroxytryptamine; 5-HT, receptor, 5-HT1f

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, A, 91/17174 (WEINSHANK ET AL.) 14 November 1991, see entire document.	1-76
A	JOURNAL OF NEUROCHEMISTRY, Volume 53, Number 2, issued 1989, Leonhardt et al., "Detection of a Novel Serotonin Receptor Subtype (5-HT1E) in Human Brain: Interaction with a GTP-Binding Protein," pages 465-471, see especially abstract.	37-47



Further documents are listed in the continuation of Box C.



See patent family annex.

Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	
"E"	earlier document published on or after the international filing date	"X"
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"
"O"	document referring to an oral disclosure, use, exhibition or other means	
"P"	document published prior to the international filing date but later than the priority date claimed	"Z"

Date of the actual completion of the international search  
25 March 1993

Date of mailing of the international search report

07 APR 1993

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231Authorized officer  
MARIANNE PORTA ALLEN  
Telephone No. (703) 308-0196

Facsimile No. NOT APPLICABLE

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/00149

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, Volume 90, issued January 1993, Adham et al., "Cloning of another human serotonin receptor (5-HT1F): A fifth 5-HT1 receptor subtype coupled to the inhibition of adenylate cyclase," see especially abstract, figures 1-4, and table 1.	1-18, 37-47, 49, 75, 76

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US93/00149

**A. CLASSIFICATION OF SUBJECT MATTER:**

IPC (5):

C12N 15/00, 15/11, 15/12; A61K 37/02, 37/04;

**A. CLASSIFICATION OF SUBJECT MATTER:**

US CL :

424/85.8; 435/6, 7.1, 69.1, 172.3, 240.2, 252.3, 252.33, 320.1; 514/12, 44; 530/350, 387.1, 388.1; 536/23.1, 23.4, 23.5; 800/2